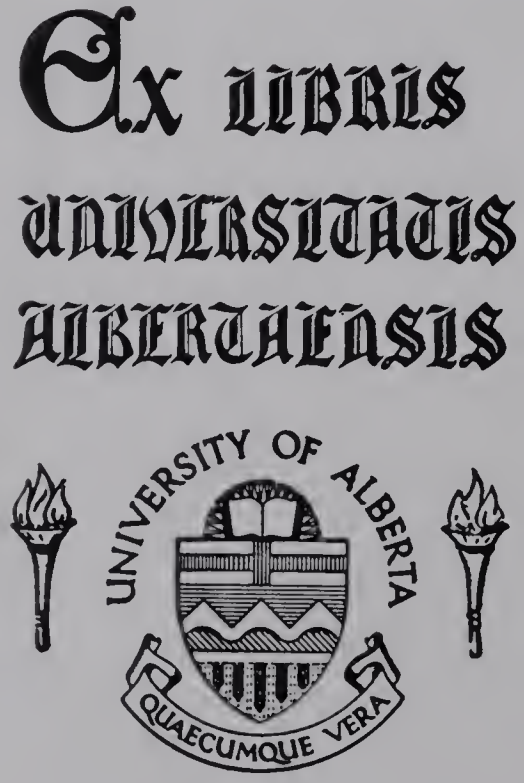

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EFFECTS OF SEVERAL SPECTRAL BANDS OF VISIBLE LIGHT ON DEVELOPMENT AND LIPID AND PROTEIN METABOLISM OF SOYBEAN SEEDLINGS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Effects of Several Spectral Bands of Visible Light on Development and Lipid and Protein Metabolism of Soybean Seedlings" submitted by Deh-Beng Ang in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Soybean seedlings were grown in darkness and in continuous light of blue, deep-red and far-red bands. Development of various parts and changes in the lipid and protein of the cotyledons and shoots were determined. Dark-grown plants had the longest hypocotyl, the shortest epicotyl and least developed leaves and root system. Deep-red-treated plants had the shortest hypocotyl, but had the best leaf and root development. The development of most plant parts in the blue and far-red bands were intermediate. However, the far-red plants were etiolated and had the greatest cotyledon expansion. As there was no difference between treatments in dry weight or water content of the plants it was reasoned that the development of different plant parts was compensatory. At termination of the experiments there was an inverse relationship between oil content of the cotyledons and cotyledon expansion. The cotyledons and embryos of the parent seed had different types and different amounts of fatty acids. During eight days of development there was practically no change within treatments, or differences between treatments, in the fatty acid composition of oil from the cotyledons. The predominant components were palmitic, stearic, oleic, linoleic, and linolenic. There were differences between treatments in the amount and types of fatty acids in the shoots. Although the percent free fatty acids in seedlings rose as the seedling developed very few were detected by GLC. Five days after treatment, cotyledons of dark-grown seedlings contained the highest amount of protein-bound amino acids and the least amount of free amino acids. Of the light-treated seedlings the far-red had the least amount of protein-bound amino acids and blue the highest. There was no difference between light treatments in the amounts of free amino acids.

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INTRODUCTION

In addition to supplying the energy for plant life, light controls certain key morphogenetic steps in the development of plants. Among these are seed germination, photoperiodism, pigment formation, stem elongation and leaf expansion. A striking feature of these phenomena is that all have been shown to be governed by the same light absorbing compound, phytochrome. As originally suggested by the Beltsville group, since the morphogenetic responses regulated by phytochrome are of such wide diversity, the action of this pigment must be at a metabolic crossing point which is common to many metabolic biochemical pathways. They reasoned that acyl activation by coenzyme A was an example of a reaction with sufficient generality to be considered as a centre for such control. Circumstantial evidence for this hypothesis was based upon the fact that anthocyanin synthesis which requires phytochrome activation is known to have acetyl CoA as a precursor in its biosynthetic pathway. Also, the germination of light-sensitive seeds is thought to require acetyl CoA as a necessary factor. A number of these seeds are small and have a high proportion of their metabolic reserves stored as fat. The degradation of fatty acids in these takes place mainly through β-oxidation activated by acyl CoA formation and the release of acetyl CoA.

If the action of phytochrome in mediating various morphogenetic effects with which it has been linked, were indeed via coenzyme A, as suggested by the Beltsville group, one should/might be able to detect differences in fat metabolism of seedlings grown under light of different quality. In this study, soybeans, in which the food reserves are mainly lipid and protein, were used as the test plant to check this possibility. The effects of several spectral bands of visible light on development and lipid and protein metabolism of the seedlings was followed.

LITERATURE REVIEW

Control of Plant Growth by Light

It is now well known that light has pronounced effects on growth and development of plants independent of photosynthesis. Many responses of plants to radiation have been recognized, among which are flowering, seed germination and etiolation. These responses, outwardly unrelated, are found to be controlled by the same photoreactions. Many photoresponses of plants and seeds have nearly the same action spectra and display similarities in photoreversibility (Hendricks and Borthwick, 1965).

Knowledge on each response has been developed independently and considerable literature has been accumulated on the research dealing with each. The present review will be restricted to etiolation, with respect to leaf enlargement, and to inhibition of elongation and unfolding of the plumular hook.

Etiolation

The word 'etiolation' derives from the French word 'etioler' meaning to grow pale or weak. It is usually used to describe the effect on growth of exclusion of light or of light deficient in certain wavelength regions.

According to Hendricks and Borthwick (1965), etiolation in plants was recognized as early as 1832 by DeCandolle. As cited by Vince (1964), MacDougal, in 1903, described differences between plants growing on their food reserves in the dark and those growing in the light, and observed effects on stem elongation, cell elongation, leaf expansion and leaf shape in many species.

Distinctive features of growth of monocotyledons and dicotyledons in darkness have been described. The leaves of monocotyledonous plants grown in darkness are long and often rolled or folded and their basal meristems maintain a capacity for continued growth. The mesocotyls or other parts of the axis are elongated. Dark-grown dicotyledonous plants often have long, weak, attenuated stems and leaves with long petioles and small blades that remain partly folded. The cells are thin-walled and loosely organized. In dark-grown dicotyledonous seedlings, there is often a plumular hook. The hypocotyls, if present are elongated.

Effect of Light on Dark-grown Seedlings

The fact that growth of many seedlings in darkness can be modified by short exposure to light of low intensities was appreciated as early as 1871 by Batalin as cited by Borthwick and Hendricks (1961). Growth responses of plants developed under a restricted region of the spectrum or a diminished intensity of light have often been described.

Light was generally found to inhibit the elongation but promote the expansion of etiolated plants or plant parts. Peas grown in complete darkness were found to be much taller than those exposed to light for several hours daily. A single short exposure to light, of etiolated pea seedlings, resulted in a decrease in stem elongation and increase in leaf growth (Went, 1941). Dark-grown red kidney bean seedlings (Downs, 1955) exhibited the typical aspects of extreme etiolation. After 10 days of growth in the dark, they were characterized by long hypocotyls, short epicotyls and very small primary leaves with short petioles and folded blades. The curvature on the upper part of the hypocotyl had disappeared but reformed a second one on the

petioles of the primary leaves. Brief exposure to incandescent light of low energy on the sixth, seventh and eighth days resulted in production of shorter hypocotyls and longer epicotyls, unfolding and marked enlargement of the primary leaves and elongation of the petioles and straightening of the hooks. The inhibition of elongation of mesocotyl (first internode) second internode and coleoptile of Avena and other grass seedlings has also been described by many authors (Avery, et. al., 1937; Inge and Loomis, 1939; Johnston, 1937; Goodwin, 1941; Weintraub and McAlister, 1942; Weintraub and Price, 1947; Goodwin and Owens, 1951; Borthwick, et. al., 1951).

By means of time-lapse photography, Galston, et. al. (1964) were able to demonstrate kinetically the effects of inductive photomorphogenically active light on etiolated peas. The effects noted include: (1) A light-induced decrease in the rate of stem elongation, beginning about 6 hours after the light treatment, and ending about 18 hours later. (2) A light-induced opening of the apical hook, beginning ca. 2-5 hours later. (3) A promotion of terminal bud growth, starting at about 4 hours after irradiation and reaching a peak about 12 hours later.

The plant organs may respond to the action of light applied directly on the organ or indirectly when applied to another structure. Avery, et. al. (1937) reported that exposure of the seeds of Avena to light for the first 10 hours of germination almost completely prevented the elongation of the first internode. In maize the elongation of the first internodes was inhibited when the tips of the coleoptiles were irradiated as they emerged from the soil (Inge and Loomis, 1937).

Response of the various organs of etiolated plants to light was found to depend on the stage of development of those parts at the time the light was given. Went (1941) observed that when illumination was given to three-day-old peas, the first internode was most shortened, the second one less, but the third and fourth were longer than in complete darkness. Illumination of four-day-old peas gave similar results, except that the first internode was hardly inhibited. For five-day-old peas, the first internode was hardly inhibited by light whereas the second internode showed the greatest decrease in length. After six days the second internode had almost completed its growth and was only slightly inhibited by the light. These observations have been confirmed by Thomson (1954), who also found a similar response in oat coleoptile (Thomson, 1951). From her results she concluded that the responses of stem or coleoptile tissue to light could be interpreted as an acceleration of the developmental process. She stated that if cells are dividing when light is given, cell division is curtailed and the transition of elongation takes place earlier; if cells are well into the elongation phase, light inhibits elongation and accelerates maturation (Thomson, 1954).

Light-influenced growth reactions in etiolated plant materials show the greatest response in the red part of the spectrum; e.g., leaf growth and internode inhibition in etiolated peas (Went, 1941; Parker, et. al., 1949); hypocotyl inhibition in bean seedlings (Withrow, et. al., 1953; Downs, 1955; Fletcher and Zalik, 1964); internode inhibition in etiolated normal and albino barley seedlings (Borthwick, et. al., 1951); mesocotyl (first internode) inhibition in Avena seedlings (Inge and Loomis, 1937; Avery, et. al., 1937; Goodwin, 1941; Weintraub and McAlister, 1942; Weintraub and Price, 1947) opening of the plumular hook in bean seedlings

(Withrow, et. al., 1957) and induction of the plumular hook in lettuce seedlings (Mohr and Noblẽ, 1960). Fletcher and Zalik (1964) using dark-grown seedlings of Phaseolus vulgaris observed that of the light-treated plants the red had the shortest hypocotyl, epicotyl and petiole with the most leaf area, while those plants exposed to the far-red region were similar to the blue treated plants which had the greatest elongation among all the light-treated plants. Went (1941), reported that of all spectral colors, green was least effective in increasing leaf growth of pea seedlings, blue produced larger leaves, and red or yellow were most effective. However, growth in length was most inhibited by the red and yellow rays, and least inhibited by blue.

Action spectra for the control of development of various growth responses of dark-grown plants or plant parts have been worked out by various investigators; e.g., for inhibition of the first internode of Avena (Weintraub and McAlister, 1942; Weintraub and Price, 1947; Goodwin and Owens, 1948) and for that of Hordeum (Borthwick, et. al., 1951); for enlargement of the primary leaves of bean (Downs, 1955) and pea (Parker, et. al.), and for promotion and inhibition of hook straightening in excised hypocotyls of bean (Klein, et. al., 1956; Withrow, et. al., 1957). The action spectra for the above mentioned growth responses were essentially the same. The regions of maximum effectiveness were found to be around 660 mμ for the red action and 730 mμ for the opposing far-red action. These wavelength regions of maximum effectiveness are thus essentially the same as those determined for the control of floral initiation (Parker, et. al., 1946; Borthwick, et. al., 1948; Parker, et. al., 1950) and germination of seeds of lettuce and Lepidium (Borthwick, et. al., 1954, Toole, et. al., 1955). The close

similarity of action spectra and repeatedly photoreversible nature of the various responses led to the isolation of phytochrome by the Beltsville group. There are excellent reviews dealing with the discovery, the purification and the properties of phytochrome (Butler, et. al., 1965; Hendricks and Borthwick, 1965; Siegelman and Butler, 1965; Siegelman and Hendricks, 1964).

The differences in elongation of petiole and hypocotyl sections of light-treated Phaseolus vulgaris seedlings were found to be more closely related to the extent of cell elongation than to an increase in cell numbers. But there was some relation between epicotyl elongation and cell numbers (Fletcher, Peterson and Zalik, 1965). The opening of the hypocotyl hook in bean seedlings was found to result from enlargement of cells on its concave side (Klein, 1959). In the leaves, the differences in growth was accounted for mainly by the differences in size of the intercellular spaces (Fletcher, Peterson and Zalik, 1965).

Biochemical Changes Related to Light

Many biochemical changes related to light and/or growth responses have been reported. Among these were anthocyanin synthesis and changes in endogenous growth substances and flavonoid content including cofactors or inhibitors of indoleacetic acid (IAA) oxidase as well as lipid and protein metabolism.

Auxin Level

Van Overbeek (1936) observed that excessive elongation of the mesocotyl was correlated with a 50% higher auxin production in coleoptile tips of plants kept in complete darkness, in comparison with plants which received occassional red and orange light. Briggs (1963a) and others

(Blaauw-Jansen, 1958) also observed that red light lowered the level of extractable and diffusible auxins from coleoptile tips. Meijer (1958a, 1958b) had suggested on the basis of his findings that IAA is involved in the effect of light on plant elongation. Recently, Fletcher and Zalik (1964) found a close correlation of elongation and IAA content of the plants, maximum inhibition and least IAA being found in the red treated plants.

Effects on Flavonoids

Galston and his coworkers observed that light affects growth through some reaction which simultaneously effects flavonoid formation. These flavonoids act either as co-factors or inhibitors of IAA oxidase, an enzyme which may govern the IAA level in the plant. Hillman and Galston (1957) observed that IAA oxidase activity in tissue or tissue hormogenates was sharply decreased by red light. This decrease in IAA oxidase activity following irradiation was due to the production of a dialyzable inhibitor (Hillman and Galston, 1957). Later, Furuya, et. al. (1962) found that the inhibitor fraction contained four flavonoid complexes, which were separated and characterized as a kaempferol-triglucoside (KG) and its coumaric acid ester (KGC), and the quercetin-triglucoside (QC) and its coumaric acid ester (QGC). The quercetin derivatives acted as inhibitors of IAA oxidase and the kaempferol derivatives acted as co-factors at low physiological concentrations. In etiolated tissue, only KG and KGC could be found in appreciable quantities, while in green tissue all four flavonoids could be detected (Furuya, et. al., 1962). Red irradiation caused an increase in flavonoid content in etiolated plumules and the promotion was negated by far-red applied immediately after the red (Furuya and Thomas, 1964). The increase in flavonoid was found to be due almost entirely to QGC (Bottomley, et. al., 1965). From these

observations they concluded that kaempferol complexes are products of etiolation metabolism while quercetin complexes are products of photo-stimulated metabolism, or to put it another way, phytochrome 660 (P_{660} or Pr) leads to the production of kaempferol, while phytochrome 730 (P_{730} or P_{fr}) leads to the production of quercetin.

The formation of anthocyanin in plants or plant tissue has also been found to be stimulated by or dependent on light and governed by the red/far-red system or high energy reaction as seen in other growth responses (Klein, et. al., 1957; Mohr, 1957; Siegelman and Hendricks, 1958). In apple skin, Siegelman and Hendricks (1958) found that absence of light prevented anthocyanin biosynthesis and resulted in accumulation of low molecular weight volatiles such as ethanol and acetaldehyde. They suggested that the effect of light was to direct acyl units into flavonoid synthesis.

Effect of Light on Lipid Metabolism

Gardner (1921) observed that embryos of various photoblastic seeds became more acid when incubated in light than when incubated in darkness. He concluded that light seems to activate lipolytic enzymes. In grass seeds, it was found that there was correlation between photoblastism and the content of neutral fats and free fatty acids. Seeds containing large quantities of free fatty acids germinated equally well in light and in darkness. Those containing more neutral fats were light-favored (Ralski, 1924). But light did not appear to influence fat hydrolysis directly because in light-favoured Poa seed and in the light-inhibited Bromus seed fat hydrolysis was fastest in light and darkness respectively (Fassbender, 1925).

Tietz (1953) claimed that the activity of lipase in Oenothera biennis was promoted by light while that in Nigella damascena was inhibited, correlating with the light requirements of those seeds.

An examination of germination in Digitalis purpurea seed was made by Grohne (1952). The tip of the radicle (root) in the dormant plantlet in the seed is immediately above the micropyle (a hole in the seed wall). An oil drop is adjacent to the micropyle. The first change observed in germination is the appearance of starch in this droplet, fat thus is being converted to starch.

A greater utilization of fat during germination or development of the seedlings in the light than in the dark has been reported by a number of authors (MacLachlan, 1936; White, 1958; Hock, et. al., 1965). Huber and Zalik (1963), however, observed that the diminution of fat in germinating flax seedlings was the same in the light as in the dark.

Some authors found that there was no preferential utilization of different fatty acid components of the storage fat (MacLachlan, 1936; Huber and Zalik, 1963). Holman (1948), however, reported a decrease in the iodine value of the fat reserve and a preferential utilization of linoleic and linolenic acid in germinating soybean seedlings. On the other hand, Brown, et. al. (1962) observed a more rapid loss of oleic acid in the cotyledon of germinating soybean seedlings. Crombie and Comber (1956) also observed that oleic acid was metabolized relatively faster than other fatty acids in germinating watermelon seedlings. White (1958), found that in germinating cotton seeds the iodine value and the percentage of linoleic acid decreased but the percentage of oleic acid increased and the percentage of saturated acids remained steady during the first four days and then dropped. He found that the depletion of the component fatty acids was the same in the unilluminated as in the illuminated plants. Huber and Zalik (1963) also observed that in germinating flax seedlings, the fatty acids were metabolized similarly in the dark and in the light.

Recently Kurtz, et. al. (1962) reported that fatty acid synthesis in flax embryos is stimulated by light.

Effect of Light on Protein

Protein synthesis has been shown to be influenced by light and may be related to the photomorphogenetic response (Vince, 1964). Ohlenroth and Mohr (1963) observed a marked increase of protein in blue light as compared with red in the protonema of ferns, and this was accompanied by the morphogenetic change associated with blue light in these plants.

In mustard seedlings Weidner, et. al. (1965) found that red light leads to an increase in protein content. On the other hand, Sisler and Klein (1961) reported that red light did not promote protein synthesis in Avena coleoptiles or in bean plumular hooks. Protein synthesis in isolated disks of potato tubers was found to depend on light (Zuker, 1963). Also, the stimulation of synthesis of phenolic compounds by light in the disks was found to depend on the light stimulation of protein synthesis.

Light has been known to have a high stimulatory effect on nitrate reduction. Stoy (1955) observed that blue light has a favorable effect on this aspect.

MATERIALS AND METHODS

Light Cabinets

The light cabinets and filters used for this study were essentially those designed by Zalik and Miller (1960). The characteristics of the cabinets and light filters had been described previously (Miller, 1965; Fletcher, 1964; Zalik and Miller, 1960).

The changes from this basic design which were incorporated consisted of (a) a light bank of 11 twenty-watt fluorescent lamps above the blue filter. The purpose of this alteration was to reduce fire hazard by stepping down from 8000 watts to 220 watts. The quality of light transmitted through this filter was still the same. (b) The copper sulphate concentration in the filter designated Deep-red was changed from 1.5 gm/l to 9 gm/l. As a consequence the peak transmittance of this filter became 660 mμ and it now does not transmit light above 700 mμ. (c) A new filter was fabricated to replace the Red-far-red filter. It was made from a filter like the one for white light, but a sheet of F.R.F. 700 plastic was placed below it. This filter does not transmit light of wavelengths below 700 mu and was designated as Far-red. Figure 1 shows the transmittance of the Deep-red and Far-red filters. The total lamp wattage used for each cabinet the color designation, the composition and the transmittance data for all the filters are included in table 1. The color designations are for convenience of reference only.

The intensity of illumination in each cabinet was adjusted to the same energy level, 2500 ergs/cm^2/sec. It was measured by an Eppley pyroheliometer of the 180° Weather Bureau type used in conjunction with a

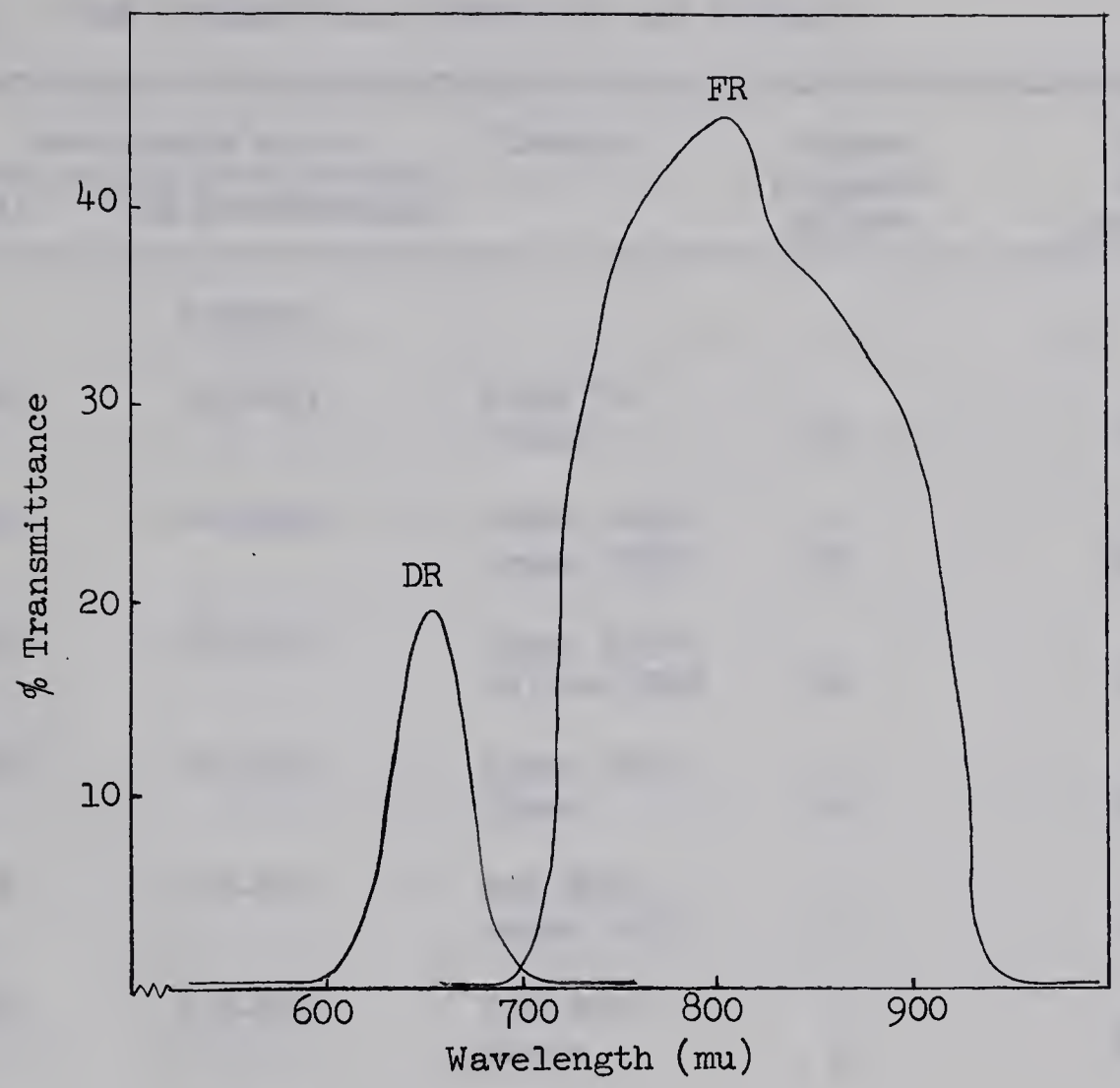


Fig. 1. Percent transmittance of Deep-red (DR) and Far-red (FR) filters.

TABLE I

Total lamp wattage, color designation, composition and transmittance data for the filters

Color	Wavelength mμ at		Plastic*	Copper sulphate GM/l**	Total lamp wattage***
	Peak	50% transmission			
Dark		control			
Blue	426	393-463	Blue 705 Clear	50	220
Green	493	460-532	Green 2082 Green 2082	75	1600
Yellow	532	500-577	Green 2154 Yellow 2208	20	1200
Orange	560	540-605	Amber 2451 Clear	10	1200
Red	600	580-655	Red 2085 Amber 2451	3	480
Deep-red	660	630-675	Red 2444 Clear	9	4000
Far-red	800	720-910	Clear Clear + R.F.R. 700	0.15	200
White	-	346-900	Clear Clear	0.15	120

* These plastics were sold under the trade names of "Perspex" (Imperial Chemical Industries Ltd.) and "Plexiglass" (Rohm and Haas Co.). The F.R.F. 700 plastic was purchased from Westlake Plastics Co., Lenni Mills, Pa.

** All solutions were made up in 0.5% sulphuric acid.

*** In all the cabinets except the blue 8 incandescent lamps were used. Eleven fluorescent lamps were used in the blue.

precision voltmeter (Nanovoltmeter, Astrodata Model TDA-121, Modification 2) having a sensitivity of 0.1 microvolt. The desired intensity was obtained by adjusting:

(1) the shelf height within the cabinets,

(2) the height of the lamp racks, and

(3) the wattage of the lamps.

Uniform temperature was maintained in all cabinets through a refrigerated, air circulating system. Heat from the lamp was dissipated by a separate air flow which was drawn over the lamps and exhausted from the room, and also by a cold water flower over the filters.

Plant Material

A variety of soybean (Soya Max), Flamebean, was used throughout these studies. This variety was chosen because of its high oil content and uniform germination. The dry weight, oil content and nitrogen content of the seed used are listed in Table II.

TABLE II

Dry weight, oil content and nitrogen content of soybean.

	Cotyledons*		Embryo		Whole seed*	
	%	mg	%	mg	%	mg
Dry weight (average of 250 seeds)		155		3.70		158.70
Total oil content	21.53	33.37	11.06	0.41	21.28	33.78
Total protein content	35.97	55.75	34.20	1.27	35.92	57.02

*Seed coats removed.

Seedling Preparation

The original bushel of seed was separated with sieves of different sizes into several lots of uniform size. The lot containing the most seeds was used for this study. The seeds were placed on wet paper towels in glass trays. They were covered with moist cheesecloth which was hung over one side of the tray and dipped into a water reservoir to draw the water continuously. The trays were placed in the dark cabinet at 23 $\pm 1^{\circ}$ C. Three days later the seeds were selected under a green safelight (Whitehouse, 1965) for uniformity of development and were transferred to 6 inch plastic pots containing a mixture of fine sand and vermiculite. About 20 to 25 seeds were planted in each pot. After an additional 2 to 3 days in the dark cabinet, the seedlings were about 1 to 3 cm tall. They were again selected for uniformity. The pots containing 15 to 20 uniform seedlings were then transferred into various light cabinets. There were 8 pots in each cabinet. Continued selection was carried out during the experiment to eliminate as much variability as possible.

All nine cabinets were used in the preliminary experiments on the effects of light quality. Based upon the results obtained the following four cabinets were chosen for the major study.

Color designation	Wavelength mμ at	
	Peak	50% transmission
Dark		
Blue	426	393 - 463
Deep-red	660	630 - 675
Far-red	800	720 - 910

The duration of light treatments used in preliminary experiments was:

A. Continuous light for 7 days.

B. 48 hours continuous light then 5 days in dark.

C. 4 hours light and 20 hours dark for 7 days.

Measurements were made on the length of roots, hypocotyls and epicotyls; volume of cotyledons, and area of leaves. On the basis of the results obtained, it was decided to use continuous light for the remaining studies.

For the major study, seedlings were grown under continuous light for 5 - 8 days. Samples of about 20 seedlings were taken before commencing the light treatment and daily thereafter. The plant materials were handled as described below.

Growth Measurements

The seedlings were separated into cotyledons and shoots and the fresh weights were determined immediately after dissection. The length of roots, hypocotyls and epicotyls were measured. The volume of the cotyledons were estimated by water displacement. After the measurements, the plant materials were frozen and freeze-dried for 2 days. The dry weights of the plant parts were determined after they were taken from the freeze dryer. The dried materials were stored over P_2O_5 in a desiccator at 4^{o} C until analyzed.

Total Lipids, Free Fatty Acid and Fatty Acid Composition

The dried materials were ground with a Wiley mill to pass a 60 mesh screen. Duplicate samples were taken for oil extraction. The samples

were brought to constant weight in a vacuum oven at 60° C overnight. The total lipid content was determined by the A.O.C.S. method (A.O.C.S. 1963). The oil samples were stored in a small amount of petroleum ether in a deep-freezer, for fatty acids analysis. The residue left after oil extraction was stored at 4° C for amino acid analysis.

The percentage free fatty acids in the oil was determined by a microtitration method (Mehlenbacher, 1960). Fifty milligrams of oil sample in 10 ml of petroleum ether and 20 ml of neutralized ethyl alcohol containing indicator (m-cresol sulfonphthalein) was titrated with 0.01 N NaOH solution to the appearance of a purple color.

The fatty acids in the neutral oil were methylated to methyl esters by transesterification, using sodium methoxide as a catalyst (Craig and Murty, 1959). The lipid solution was dried over anhydrous sodium sulfate before methylation. The reaction failed to complete if there was any moisture or excessive free fatty acid present. The latter was removed by column chromatography (Carroll, 1961). One hundred µl of reagent containing 0.5 gm of sodium metal in 1 liter of anhydrous methanol was added to 0.01 gm of oil in a serum vial equipped with rubber septums. The mixture was kept in the oven at 60 - 70° C for 1 - 2 hrs. Methylation was considered complete as shown by a clearing of the system which was initially in two phases. The methyl esters formed were injected immediately without further treatment into the gas-liquid chromatography column. This was done to reduce further loss of the volatile and water-soluble methyl ester of short chain fatty acids if present.

The free fatty acids were separated from the oil by column chromatography on Florisil (Magnesium silicate) using a method similar to that described by Carroll (Carroll, 1961). A 20 gm column, measuring

1.9 x 16 cm was used. The amount of oil applied for each separation was 150 mg. The elution was done with 150 ml of 50% diethyl ether in hexane for diglycerides, triglycerides and sterols; 100 ml of 2% methanol in diethyl ether for monoglycerides and 100 ml of 4% acetic acid in diethyl ether for free fatty acids. The free fatty acid fraction was dried over anhydrous sodium sulfate and the solvent evaporated on a rotary flash-evaporator at room temperature. The fatty acids were then methylated by a BF_3 method (Metcalfe and Schmitz, 1961). One ml of reagent, containing 125 gm of BF_3 in 1 liter of anhydrous methanol, was added to the fatty acids, separated from 150 mg of oil, in a screw-cap test tube. The mixture was boiled for 2 minutes in a steam bath. The methyl esters formed were injected without further purification into the gas-liquid chromatography column.

The fatty acid esters were analyzed on an Aerograph Model 204 gas chromatograph equipped with an hydrogen flame ionization detector. A coiled copper column, 12 ft. long by 1/8 in. O.D. packed with 12% DEGS (diethylene glycol succinate) on 100/120 mesh chromosorb W (obtained from Applied Science laboratories, Inc., State College, Pa.) was employed. The operating conditions were: column temperature 190° C, injector temperature 240° C, detector temperature 255° C and flow rate of carrying gas (N_2) 20 ml per minute. Two to five µl of sample was injected with a constant rate syringe. At least 3 analyses were made for each sample. The peaks were recorded on a Beckman Model 1005 ten-inch laboratory potentiometric recorder operating at 1 MV range. Chart speed was 0.5 in. per minute. The peaks were identified by matching known fatty acids with unknowns. The highly purified fatty acids methyl ester standards were obtained from Applied Science Laboratories, Inc., State College, Pa. The weight percentage of

the fatty acid esters in the samples was calculated by measuring the peak area as 1/2 base times height.

Nitrogen Determination

The total nitrogen content of the dry materials was determined by a microkjeldahl method using potassium sulfate and selenium as catalyst (A.O.A.C. 1955). Protein content was expressed as total nitrogen x 4.93.

Amino Acids and Amides Analysis

Amino acids and amides analyses were carried out only for cotyledons separated from seedlings taken on the fifth day of the continuous light treatment.

The oil-free materials were ground with a mortar and pestle. One to 300 mg of sample were extracted three times with 30 ml of 95% ethanol in a 50 ml stainless steel centrifuge tube. The extract was cleared by centrifugation at 20,000 xg for 10 minutes. The three supernatants for each sample were combined and the solvent was evaporated to dryness on a rotary flash-evaporator at room temperature. Ten ml of water were then added to the flask. The solution was washed several times with diethyl ether. The ether was siphoned off with a fine capillary tube attached to a vacuum flask. The water solution which remained in the flask was flash evaporated to dryness at room temperature. The sample was made up to volume with a mixture of 1:1 distilled water:"sample diluting buffer" (Miller, 1965). The final pH was 2.2. The amino acids in this extract were taken as the "free" amino acids of the sample.

The pellets left after the ethanol extraction were air dried and hydrolyzed for 36 hours with constant boiling HCl. The digests

were flash evaporated to dryness at 80° C. The residue was taken up with 10 to 20 ml of water. The pH of the solution was brought to 2 - 4 using NaOH. Ten to 20 ml of sample diluting buffer were added and the sample made to suitable volume with 1:1 water:sample diluting buffer. The final pH was 2.2.

To determine the free amides in the extracts 1 ml of aqueous sample from the ethanol extract was hydrolyzed with 1 ml of 2 N HCl for 3 hours. The acid was neutralized with 0.5 ml 4 N NaOH after hydrolysis. The solution was then freeze-dried and the sample made up to volume with a 1:1 water:sample diluting buffer. The amide content was determined by comparing the aspartic and glutamic contents before and after hydrolysis. Also the content of free threonine and serine which overlap with asparagine and glutamine could be determined after hydrolysis.

The amino acids prepared by the above procedures were analyzed on a Model 120 Spinco Amino Acid Analyser, with a 50 cm column of Beckman type AA-15 cation exchange resin for acidic and neutral amino acids and a 10 cm column of Beckman type AA-27 resin for basic amino acids.

RESULTS

Effect of Light Duration and Light Quality on Growth and Development of Soybean Seedlings

Experiments involving different durations of light treatment in combination with the various wavelengths of light were conducted to select the light duration for use in the major study. The results obtained are illustrated in figure 2. Of the three durations used, continuous light (duration A) appeared to result in maximal differences between the effects of light of different quality (Fig. 2). However, statistical analysis showed that the effect due to light duration was significantly different only for leaf expansion. Also, the interaction between light duration and light quality was significantly different for root elongation and cotyledon and leaf expansion. Far-red was more effective than deep-red in cotyledon expansion with continuous light (duration A), whereas, with 48 hours continuous light followed by dark (duration B), or cycles of 4 hours light and 20 hours dark (duration C), deep-red was more effective (fig. 2a). For blue light durations A and C were equally effective in promoting cotyledon expansion (fig. 2a). The same trend in leaf expansion was observed for all three light durations, with deep-red being the most effective and far-red least effective (fig. 2b). Of the three durations used, duration A resulted in the greatest leaf expansion and duration B the least. There were minor differences in elongation of hypocotyls, epicotyls and roots due to different durations of light (Fig. 2c, d and e). Deep-red was most effective in inhibiting hypocotyl elongation in all three light durations (Fig. 2c). With duration A, blue and far-red were equally effective while with duration C greater inhibition was found in the blue than in the far-red and the

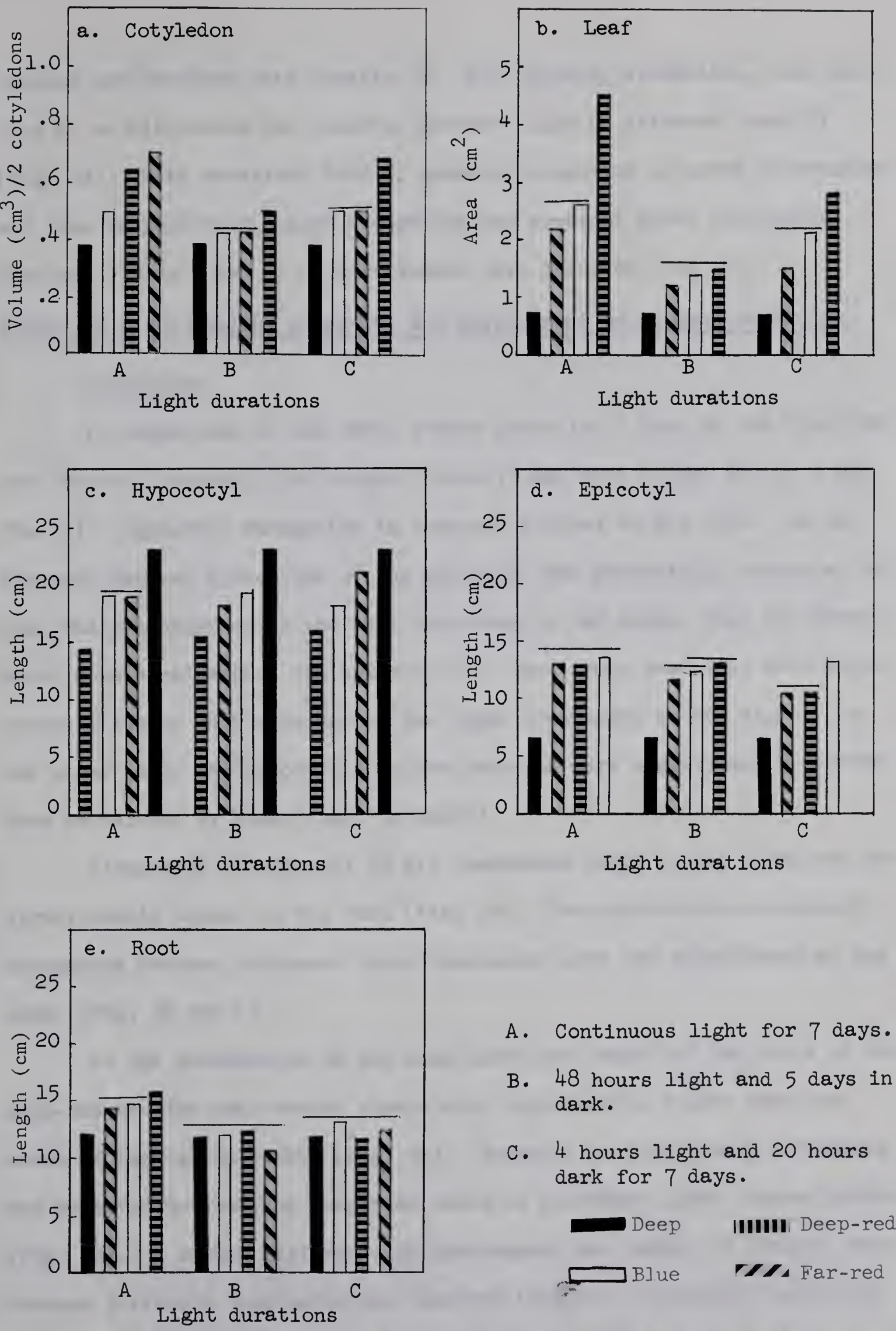


Fig. 2. Effect of light quality and light duration on expansion of cotyledon and leaf, and elongation of the epicotyl, hypocotyl and root of soybean seedlings. Each bar represents an average of two experiments. Bars not capped by the same line indicate statistically significant differences.

reverse was obtained with duration B. For epicotyl elongation, with duration A, no difference was observed between light of different quality (fig. 2d). With durations B and C, greater elongation occurred in deep-red and blue respectively. Root elongation was greatest under continuous deep-red. With B and C, no differences were observed (fig. 2e).

Effect of Light Quality on Growth and Development of Soybean Seedlings

Elongation

In comparison to the dark, plants grown for 8 days in the light had the shortest hypocotyl but longest epicotyl and root (figs. 3a, b, c and fig. 4). Hypocotyl elongation in deep-red stopped at day five. In the blue and far-red elongation of the hypocotyl had essentially ceased at day six, while elongation in the dark continued to day eight, when the experiments were terminated. The hypocotyls of dark-grown seedlings were significantly longer than from any of the light treatments at day eight. On the other hand, the hypocotyls in the deep-red were significantly shorter than in far-red or blue (figs. 3a and 4).

Elongation of epicotyl in all treatments began on day three but was significantly slower in the dark (fig. 3b). The differences in epicotyl elongation between different light treatments were not significant at day eight (fig. 3b and 4).

At the termination of the experiment the length of the roots of the deep-red and far-red treated plants were signficantly longer than the roots of dark-grown plants (fig. 3c). However, no significant difference was observed between the length of roots of different light treated plants (fig. 3c). A marked difference in development and number of lateral roots between different treatments was observed (fig. 5). Although counts and

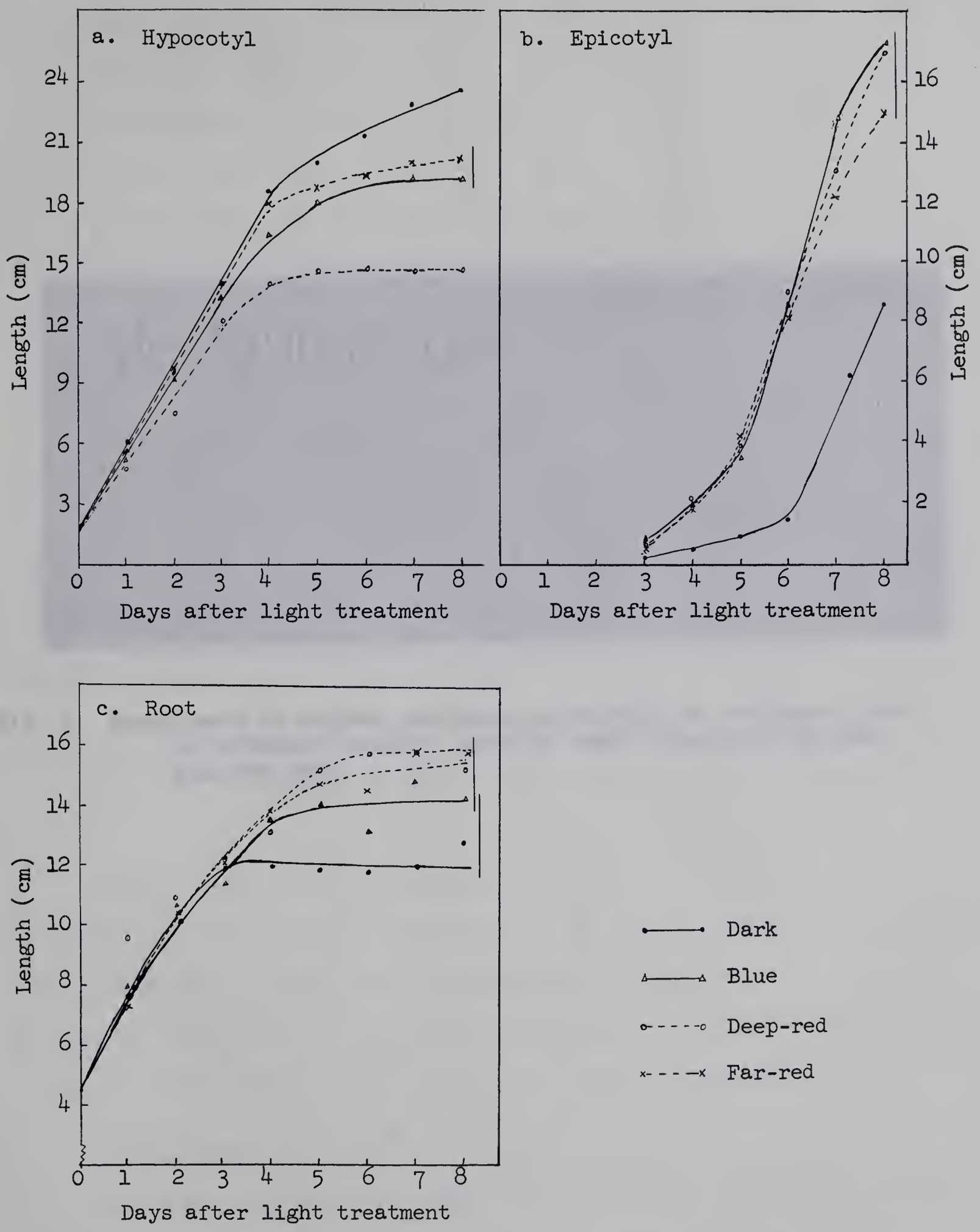


Fig. 3. Elongation of hypocotyl, epicotyl and root of soybean seedlings over the period of 8 days in the dark or under continuous light of different quality. Each point represents the mean of two experiments. Points not joined by the same vertical line indicate results for the eighth day that were significantly different.

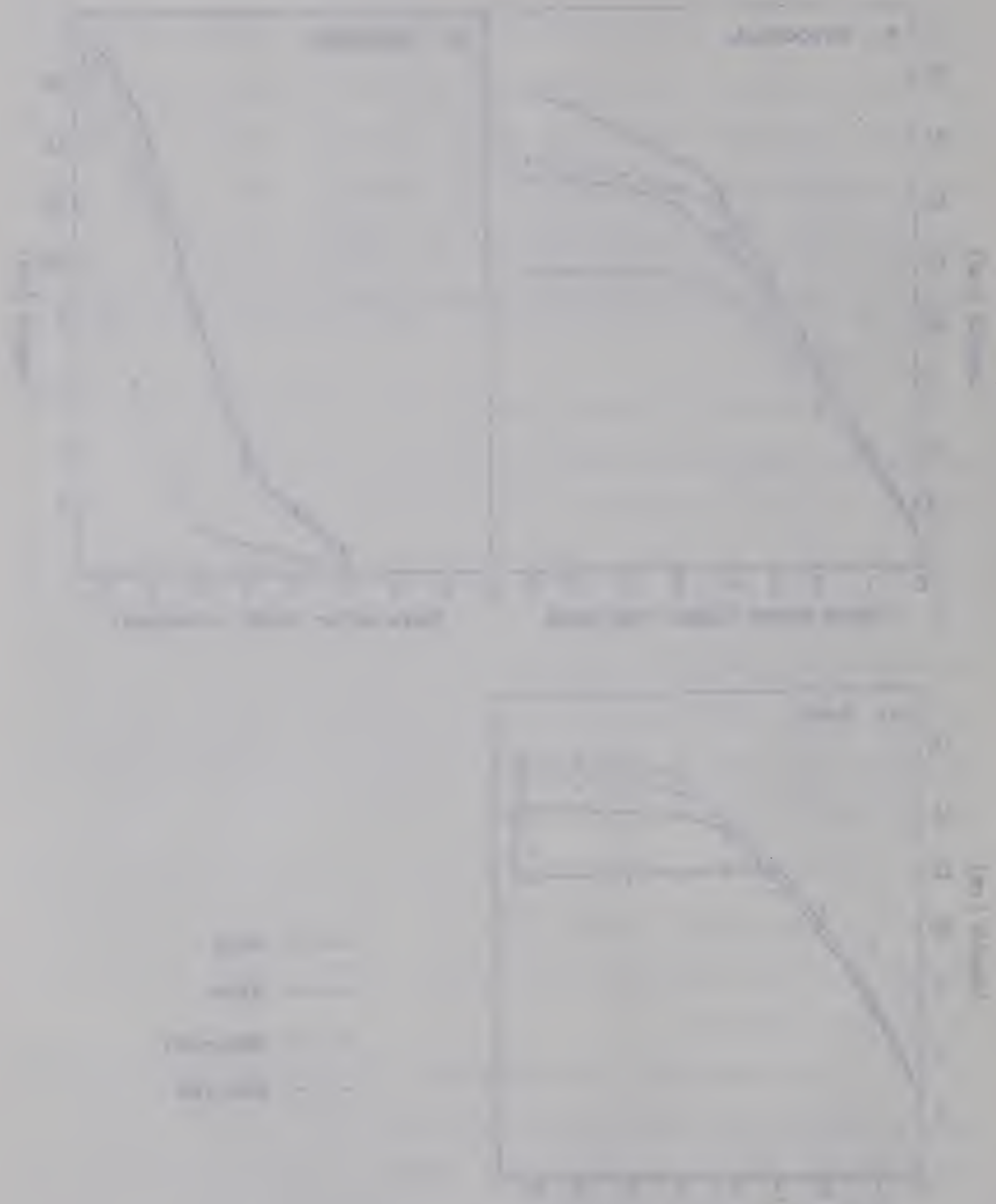


Fig. 4. Development of soybean seedlings in the dark or continuous light of different quality. Left to right: Deep-red; Far-red; Blue and Dark.

measurements of lateral roots were not made the length and number on dark-grown plants were reduced in comparison to light treated plants. Of the light treated plants the deep-red appeared to have the most and longest lateral roots. These observations were supported by the data in table III. It may be seen that there was significantly better root development in the deep-red than for the other treatments, and the poorest development occurred in the dark.

Expansion

The expansion of the cotyledon over the period of 8 days under continuous light of different spectra is illustrated in fig. 6. Practically no expansion of cotyledons of dark-grown plants occurred during the experiment, whereas the cotyledons of the light treated plants continued to expand throughout this period. At the end of the experiment, expansion of cotyledons was significantly different between all treatments. Although, the cotyledons from both the far-red and dark treatments lacked chlorophyll the far-red had the largest cotyledons and the dark the smallest (figs. 6 and 7).

Figure 7 also shows the development of leaves after eight days of treatment. The leaves failed to develop in the dark and the fullest development occurred in the deep-red. The measurements of leaf areas in separate experiments showed that the differences in leaf expansion were significantly different for all treatments as shown by the data in table III.

Water Content

The water content in all parts of the seedlings increased throughout the period of study. In cotyledons, the water content increased very slowly, whereas the water content in the shoots increased rapidly (fig.

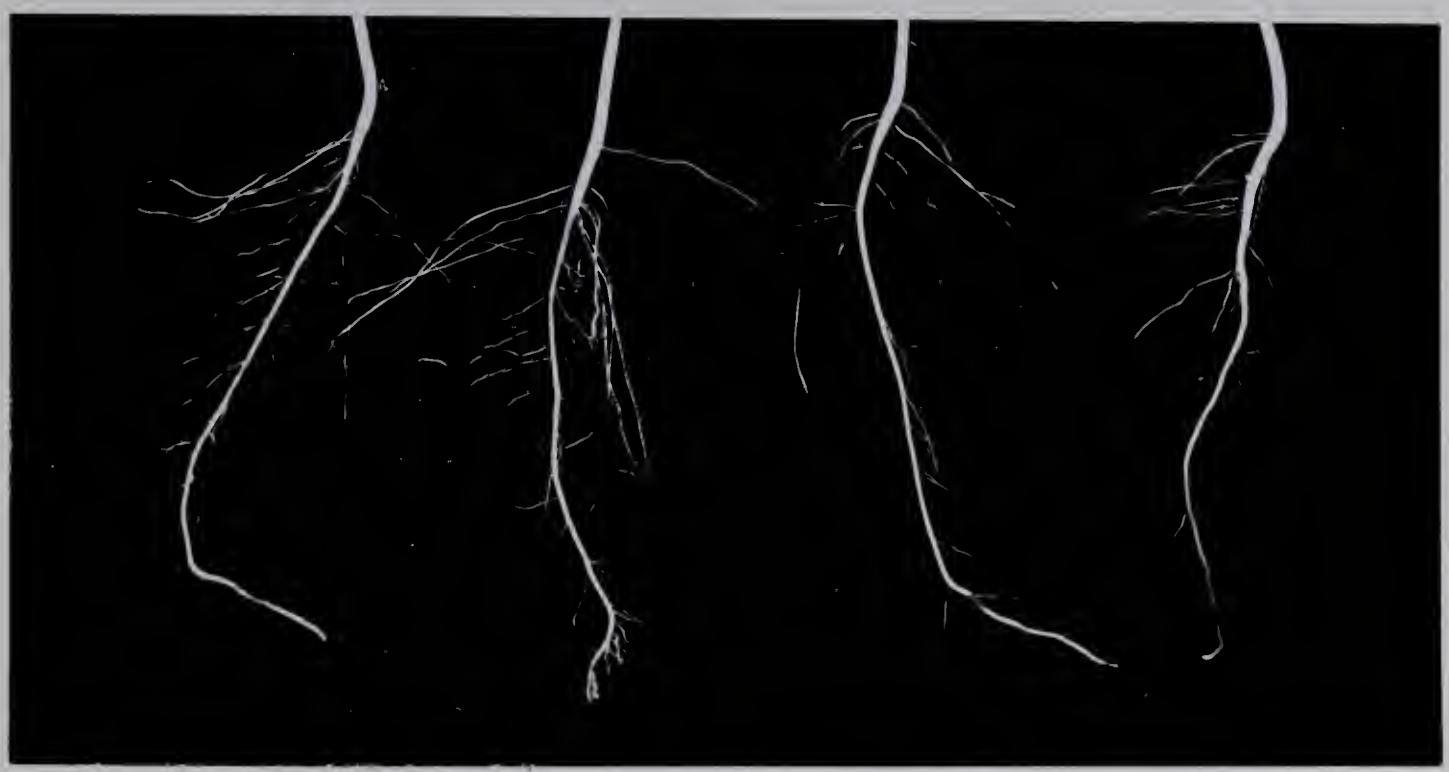


Fig. 5. Development of roots of soybean seedlings grown for eight days in the dark or continuous light of different quality. Left to right: Far-red; Deep-red; Blue and Dark.

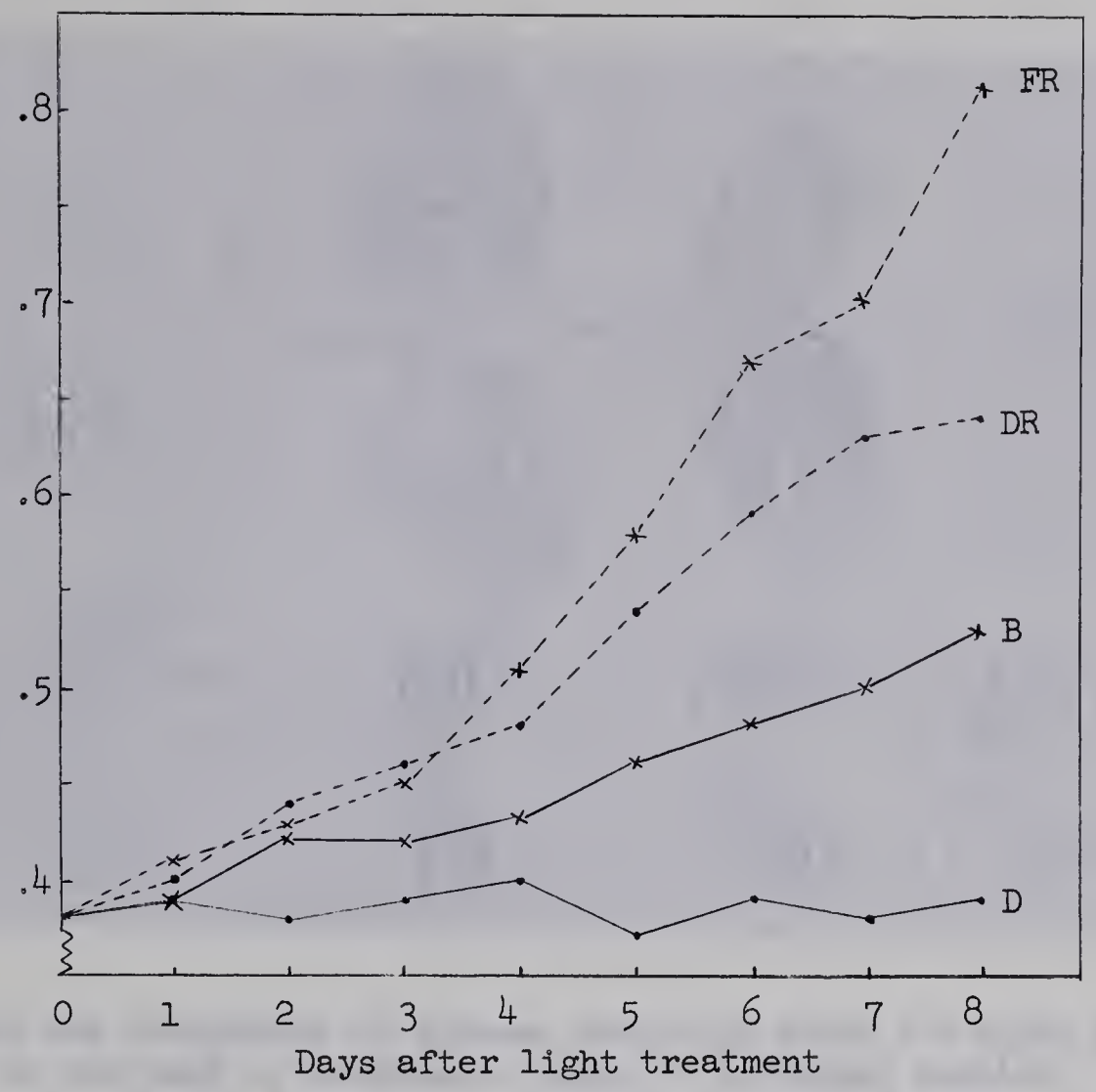


Fig. 6. Expansion of cotyledons (cm^3) of soybean seedlings over the period of 8 days in the dark or under continuous light of different quality. FR = far-red; DR = deep-red; B = blue; D = dark. Each point represents the mean of two experiments. Points not joined by the same vertical line indicate results for the eighth day that were significantly different.

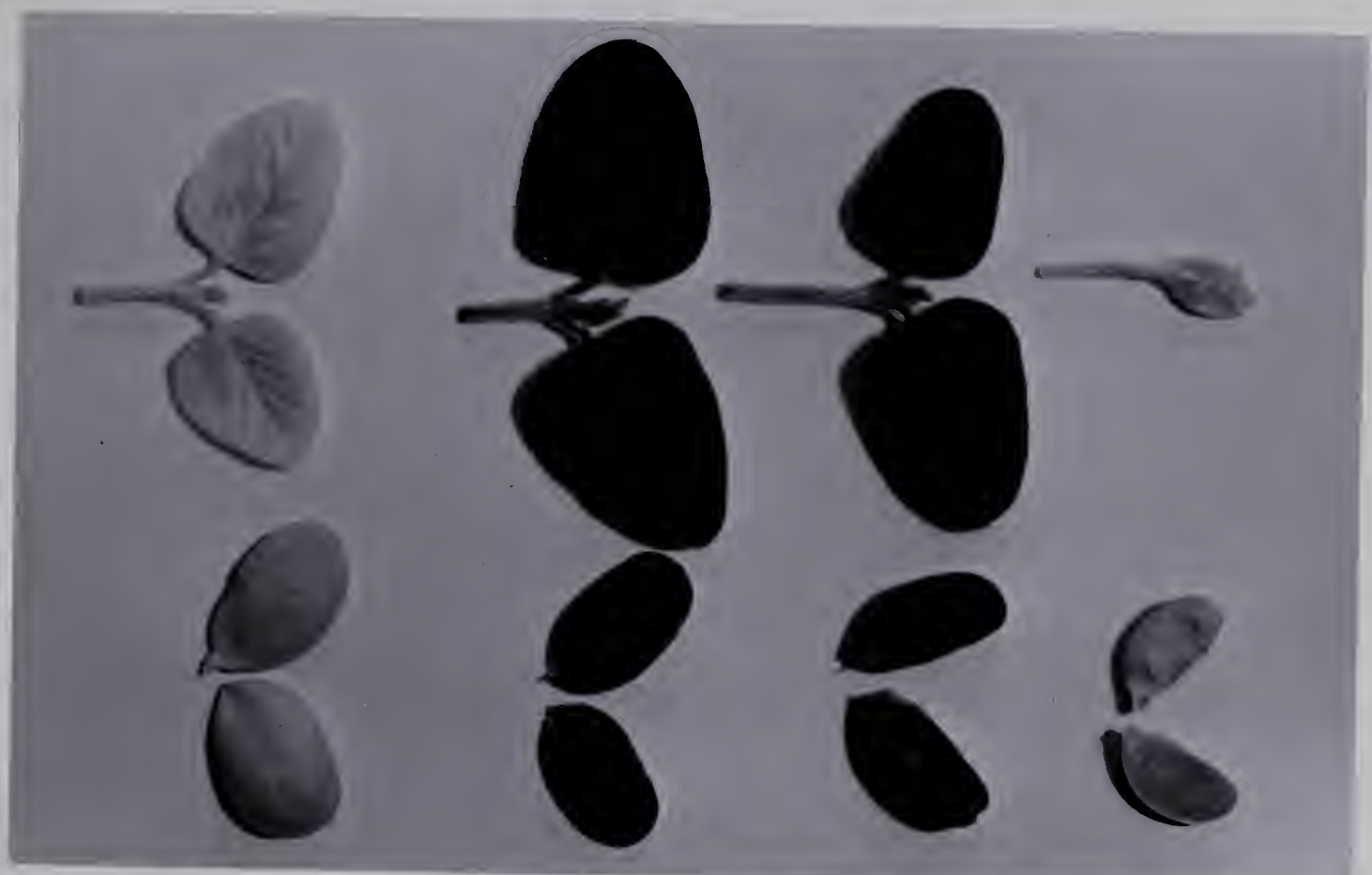


Fig. 7. Leaves and cotyledons of soybean seedlings grown for eight days in the dark or continuous light of different quality. Upper row - leaf. Lower row - cotyledon. Left to right: Far-red; Deep-red; Blue and Dark.

TABLE III

Dry weight of roots and leaf area of soybean seedlings grown for 8 days in the dark or under continuous light of different quality

Light Treatment	Dry Weight mg/root	Leaf Area (cm^2)
Dark	9.00 c	0.81 d
Blue	12.50 b	3.30 b
Deep-red	17.50 a	4.70 a
Far-red	11.58 b	2.36 c

The results are an average of 2 experiments. Figures in the column followed by different letters are significantly different at the 5% level.

8a and b). There was essentially no difference between treatments in water content of the intact seedlings during the first three to four days of growth. However, by the fifth and sixth days differences were apparent between the dark-grown seedlings and the others (fig. 8c).

Dry Weight

During the experimental period there was a decrease in dry weight of the cotyledons (fig. 9a). This was accompanied by a continuous gradual increase in the dry weight of the shoots for all treatments (fig. 9b). The shoots of dark-grown seedlings did not increase in dry weight after the fourth day. Although elongation and expansion of the shoot and cotyledon were observed (figs. 3 and 6) there was, in fact, a slight decrease in dry weight of the whole seedlings during the experiment (fig. 9c). However, there was not a significant difference in dry weight of cotyledons, shoots or whole seedlings between treatments.

Effect of Light Quality on Lipids of Soybean Seedlings

Lipid Content

Determinations of oil content in cotyledons and shoots of soybean seedlings grown under light of different spectra and in the dark were done for six experiments. Sufficient plant material for analyses of various parts at each stage were available only for the experiment reported in fig. 10. However, analyses of the other experiments gave results in close agreement with these.

The changes in lipid content of plants grown in the dark and under different light spectra produced similar patterns. There was a continuous decrease in the percent oil content and in mg oil in the cotyledons and whole seedlings during the experimental period (figs. 10a, b, e, f).

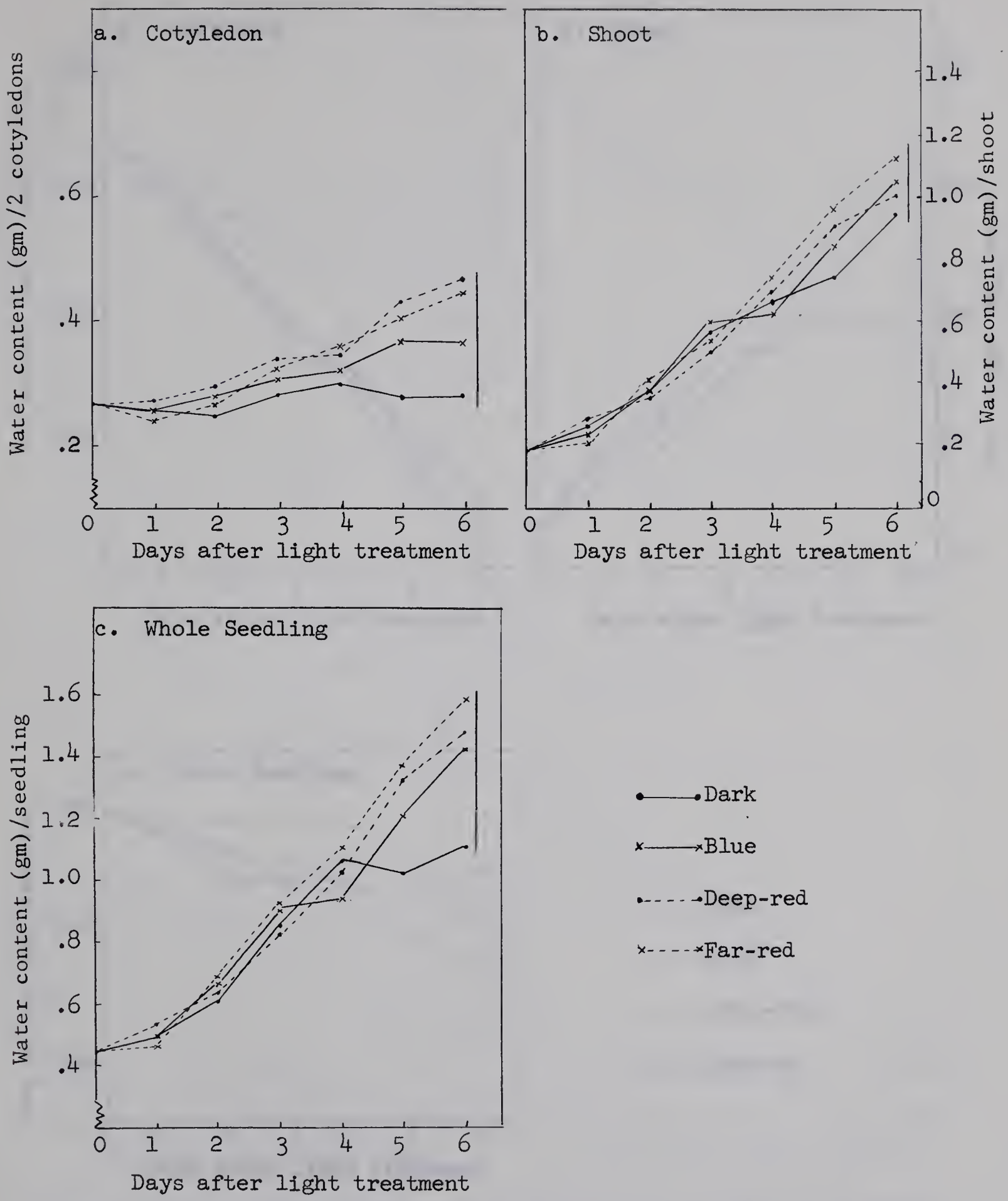
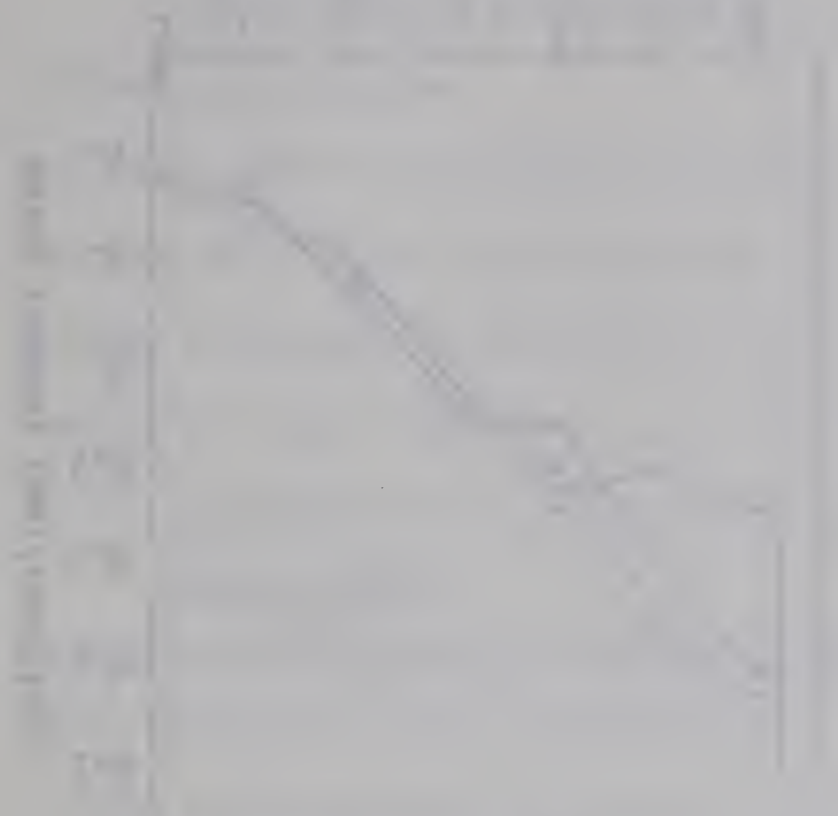


Fig. 8. Water content of cotyledons, shoot and whole seedling of soybean during the period of 6 days in the dark or under continuous light of different quality. Points not joined by the same vertical line indicate results for the sixth day that were significantly different.



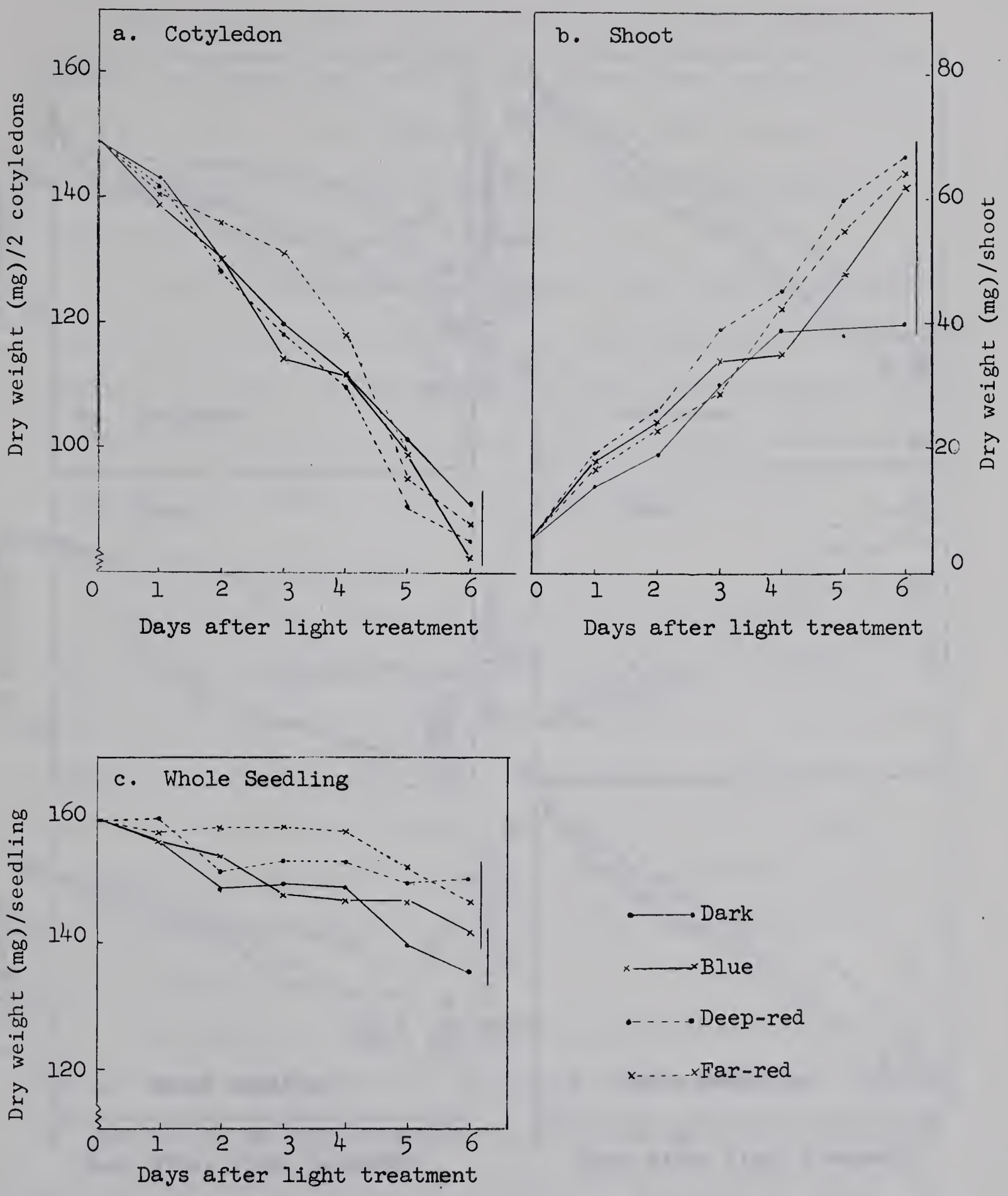


Fig. 9. Dry weight of cotyledons, shoot and whole seedling of soybean over the period of 8 days in the dark or under continuous light of different quality. Each point represents the mean of two experiments. Points not joined by the same vertical line indicate results for the eighth day that were significantly different.

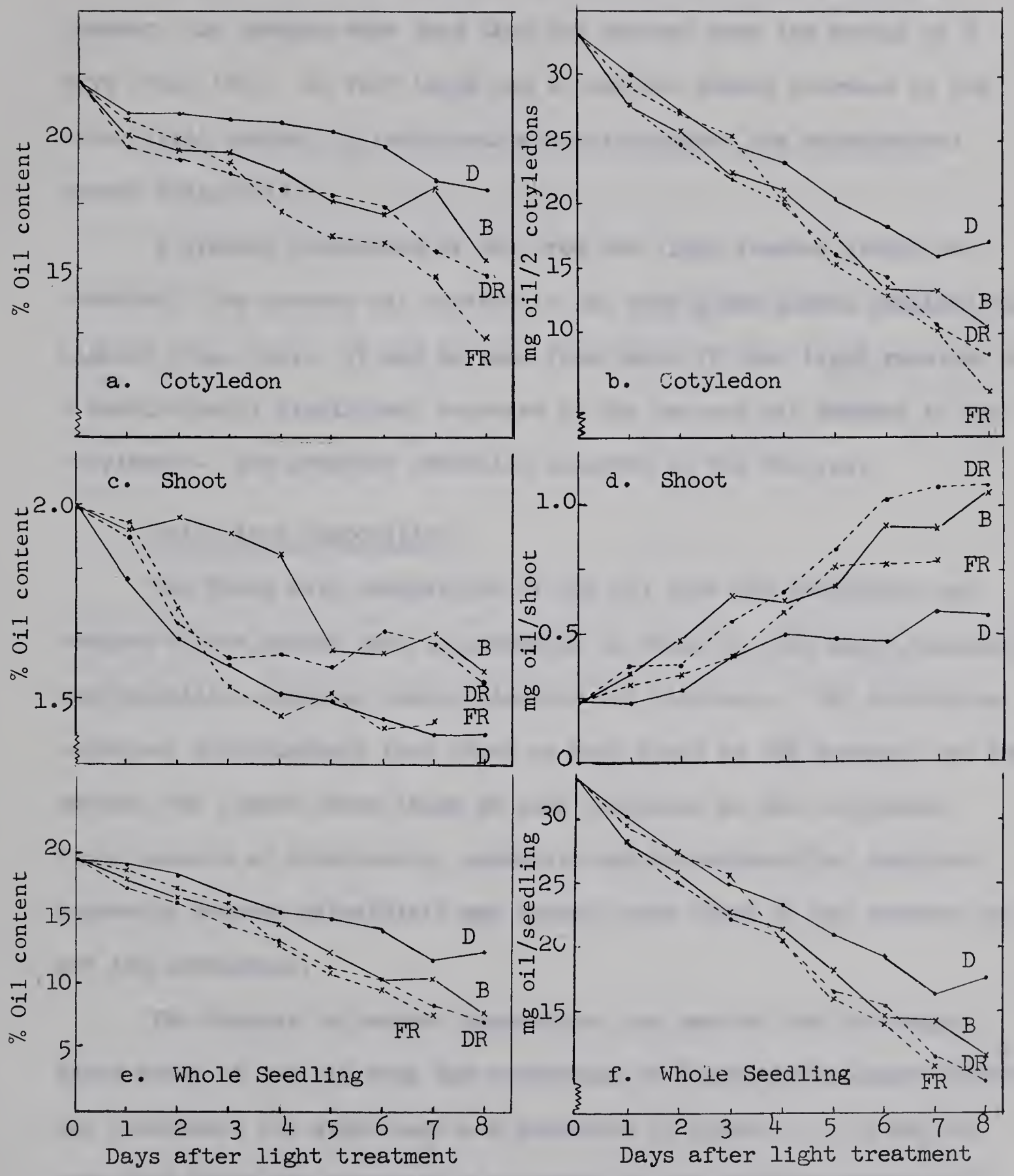
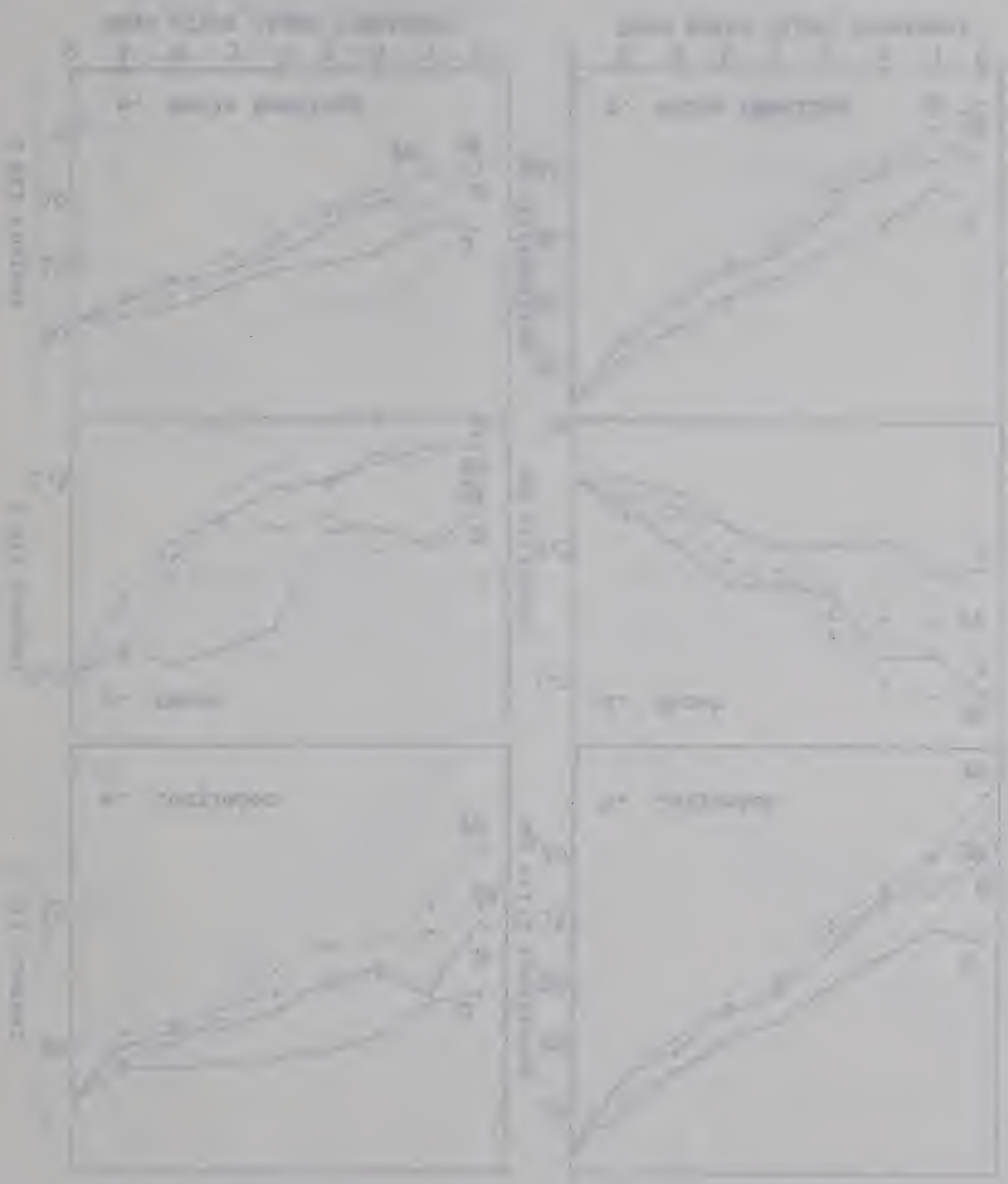


Fig. 10. Oil content of the shoot, cotyledons and whole seedling of soybean during 8 days in the dark or under continuous light of different quality. The data are expressed as percentage of dry tissue in a, c and e and as weight of oil per plant part or per seedling in b, d and f. D = dark; B = blue; DR = deep-red; FR = far-red.



The percent lipid content in the shoot decreased gradually, however, the changes were less than one percent over the period of 8 days (fig. 10c). In fact there was a slow but steady increase in the total lipid content of individual shoots throughout the experimental period (fig. 10d).

A greater diminution of oil from the light treated plants was observed. The percent oil content in the dark-grown plants remained the highest (fig. 10e). It may be seen from table IV that light resulted in a statistically significant decrease in the percent oil content in the cotyledons. The greatest reduction occurred in the far-red.

Fatty Acid Composition

The fatty acid composition of the oil from the cotyledons and embryos of the parent seed is presented in table V. The major components were palmitic, stearic, oleic, linoleic and linolenic. The cotyledons contained approximately four times as much oleic as the embryos, but the embryos had almost three times as much linolenic as the cotyledons. Trace amounts of palmitoleic, arachidic and an unidentified component appearing between palmitoleic and stearic were found in the embryos but not the cotyledons.

The changes in percent composition for each of the five major fatty acids of the oil from the cotyledons of plants grown under different treatments for eight days are presented in figure 11. It may be seen that there was essentially no change in the relative amounts of individual fatty acids and there was no difference between treatments during the experimental period.

The fatty acid composition of the lipid from the shoots changed somewhat with the development of the seedling (fig. 12 and table VI).

TABLE IV

Percent oil content in cotyledons of soybean seedlings grown for 6 or 8 days in the dark or continuous light of different quality

Treatments	Percent Oil Content*	
	6th day	8th day
Dark	18.84a	17.25a
Blue	16.95 b	15.46ab
Deep-red	17.29ab	14.64 b
Far-red	14.95 c	12.03 c

*Percent oil is expressed on dry weight of tissue. The results for the 6th day are the mean of 4 experiments, for the 8th day the mean of 2 experiments. Figures in the column followed by different letters are significantly different at 5% level.

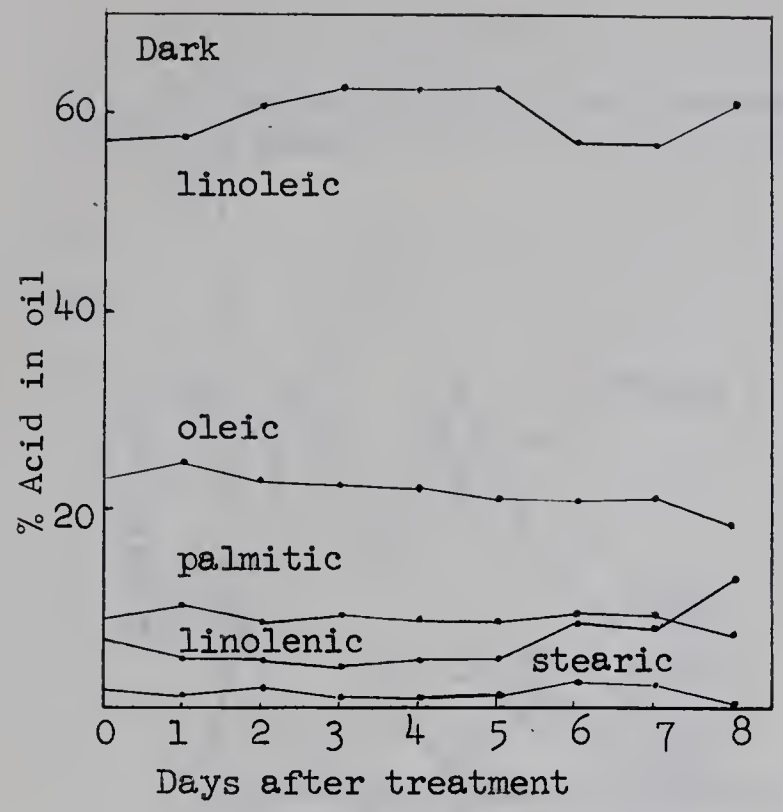
TABLE V

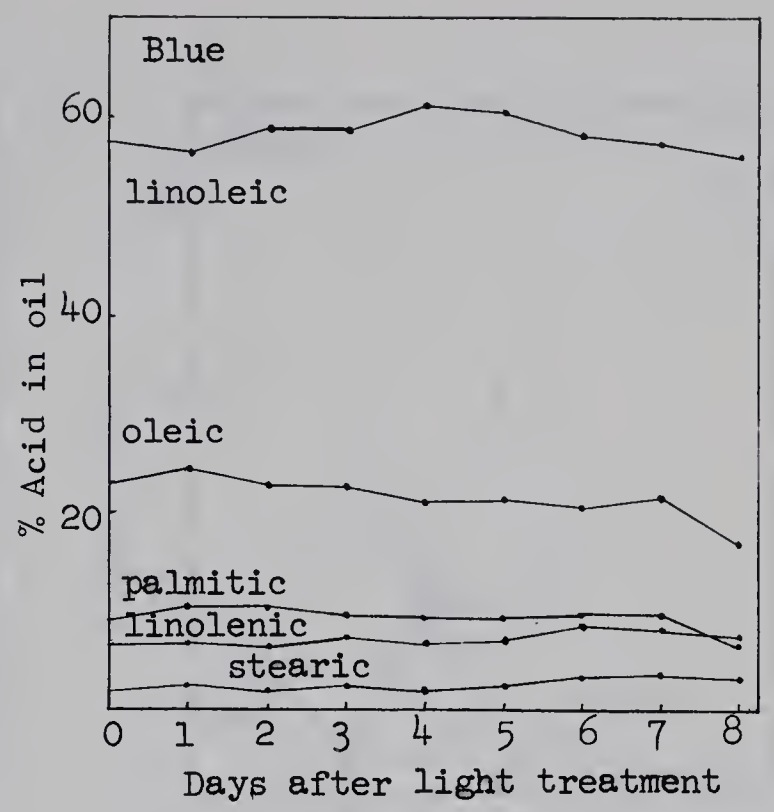
Fatty acid composition of the oil from cotyledons and embryos of ungerminated soybeans

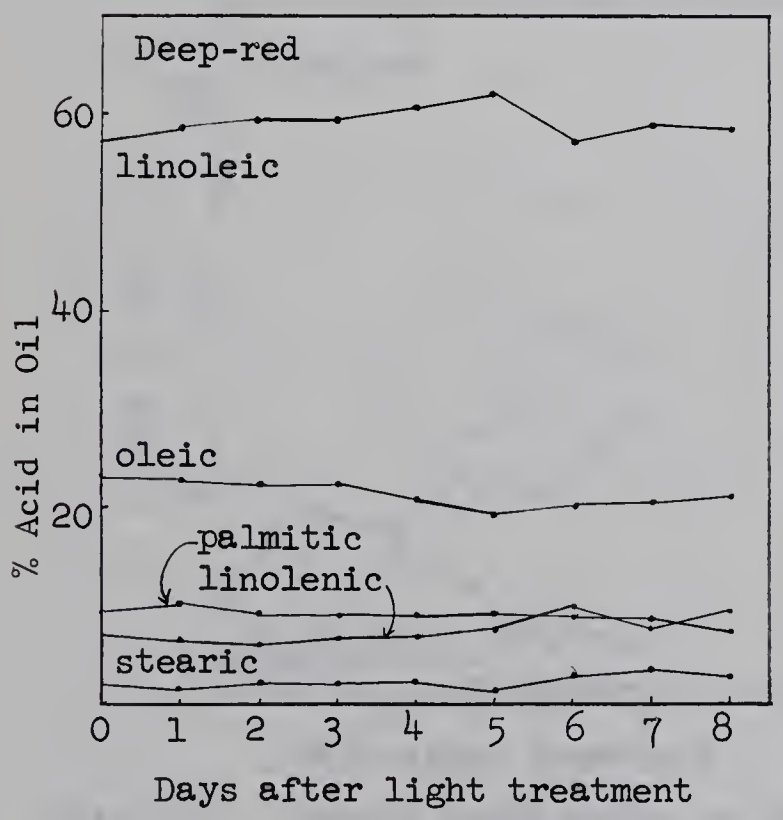
Fatty acid	% Composition	
	Cotyledon	Embryo
Caprylic $C_{8:0}$	T	T
Lauric $C_{12:0}$	T	T
Myristic $C_{14:0}$	T	T
Palmitic $C_{16:0}$	10.52	13.68
Palmitoleic $C_{16:1}$	–	T
Unknown x $C_{16:2}$'?)	–	T
Stearic $C_{18:0}$	1.77	1.89
Oleic $C_{18:1}$	23.57	5.50
Linoleic $C_{18:2}$	57.42	60.18
Linolenic $C_{18:3}$	6.73	18.76
Arachidic $C_{20:0}$	–	T
Unknown Y	T	T
Unknown Z	T	T

T = Trace

– = Not detected







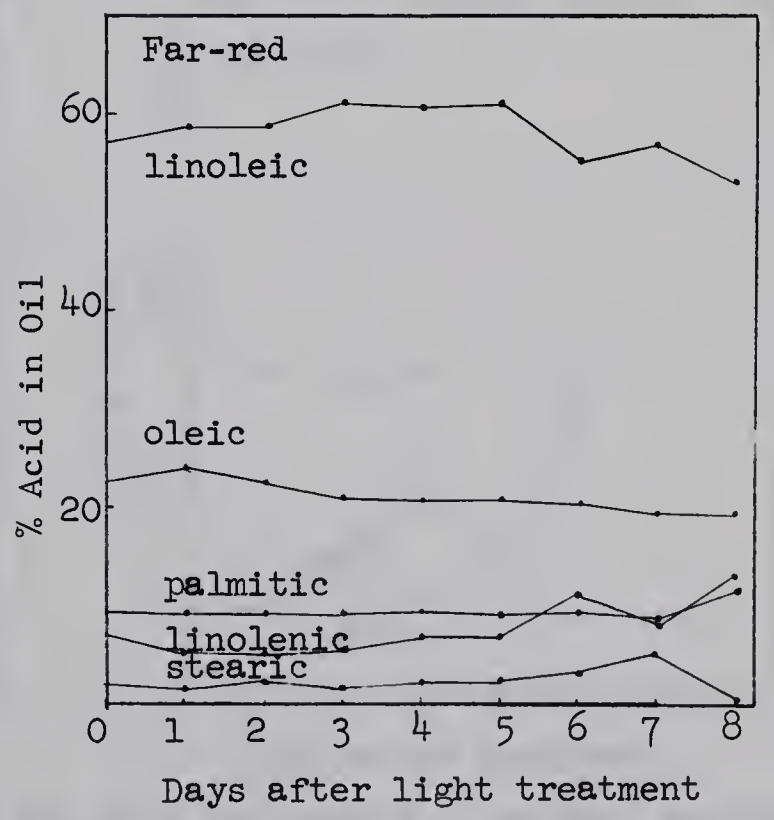
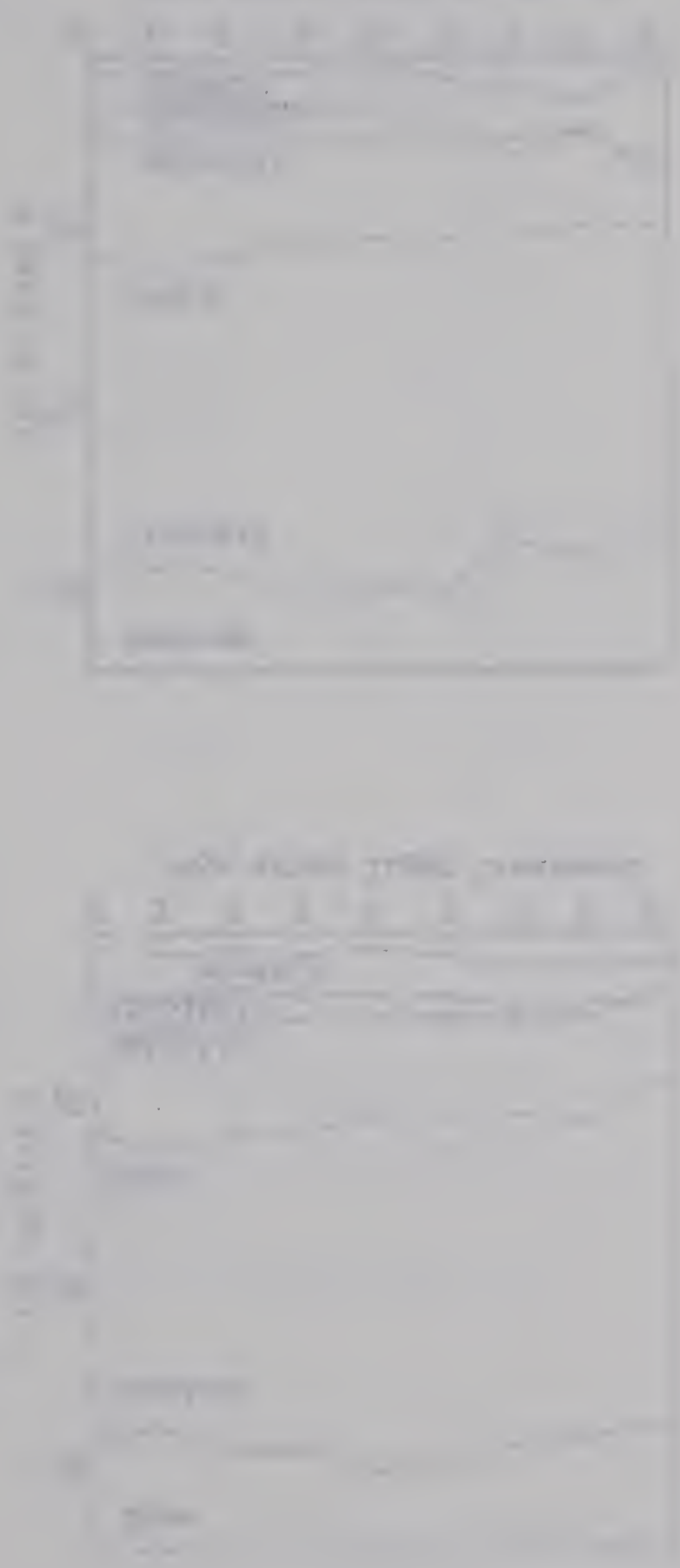


Fig. 11. Percent fatty acids in the oil from the cotyledons of soybean seedlings during 8 days of development in the dark or under continuous light of different quality. From day 0 to day 5, each point represents the mean of two experiments; from day 6 to day 8, each point represents the mean of duplicate analyses of oil from a single experiment.



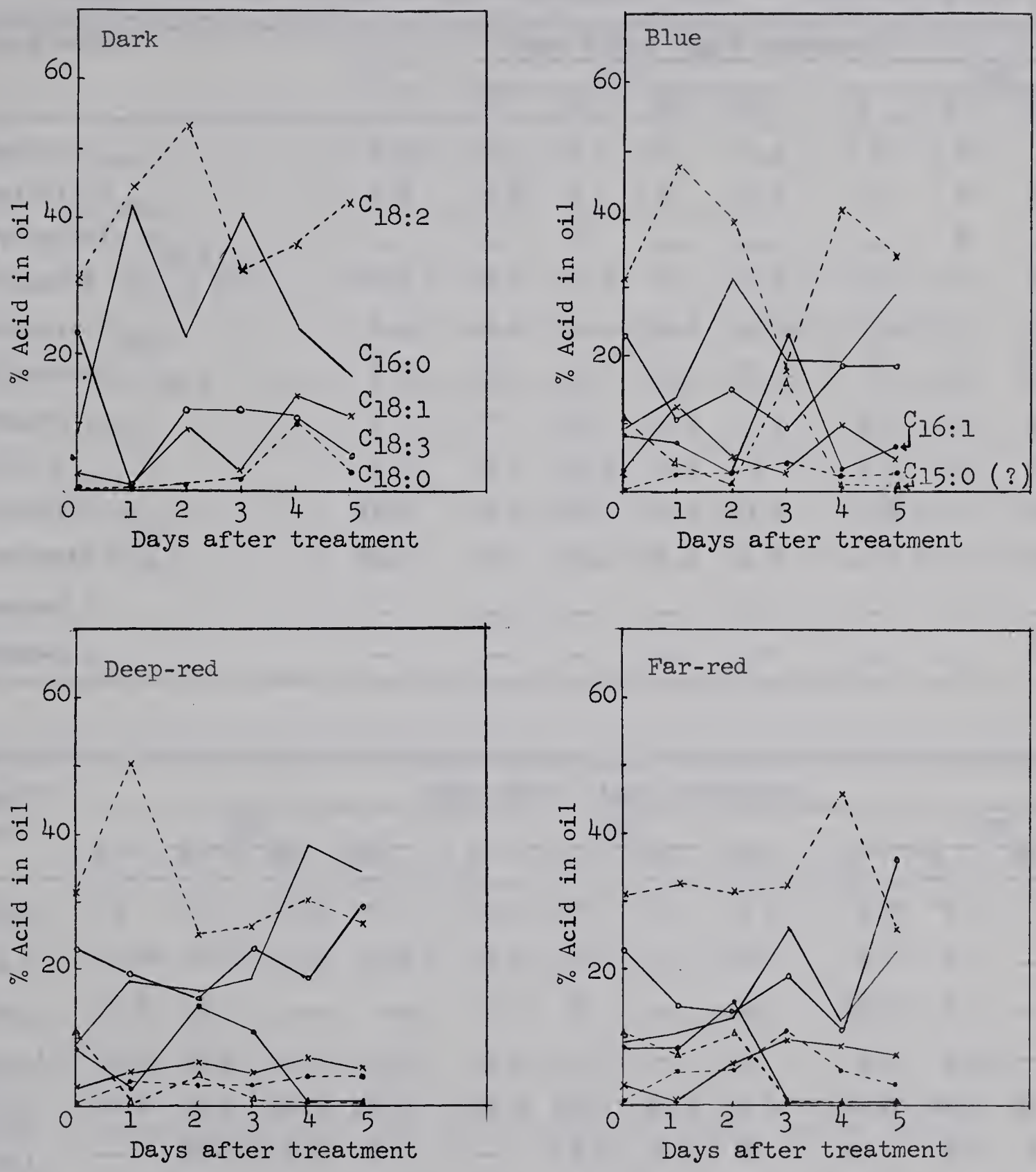


Fig. 12. Percent fatty acids in the oil from the shoots of soybean seedlings during growth for 5 days in the dark or under continuous light of different quality.

TABLE VI

Fatty acid composition of the oil from the shoots of soybean seedlings during five days in the dark or continuous light of different quality

Fatty acid	Days after light treatment								
	0	1st				2nd			
		D	B	DR	FR	D	B	DR	FR
Lauric $C_{12:0}$	4.6	2.1	T	T	4.9	T	T	—	2.5
Myristic $C_{14:0}$	7.8	5.8	T	T	5.7	T	T	2.6	2.1
Myristoleic $C_{14:1}$	—	—	T	—	—	—	T	—	—
Unknown X $C_{15:0}$ (?)	10.5	6.2	4.3	T	7.5	T	1.7	4.1	10.1
Palmitic $C_{16:0}$	9.5	41.6	14.0	18.2	10.15	23.4	31.3	17.3	12.9
Palmitoleic $C_{16:1}$	9.2	T	7.6	2.6	8.7	T	3.5	15.1	15.8
Stearic $C_{18:0}$	T	T	2.9	3.2	4.7	1.1	3.2	3.1	5.3
Oleic $C_{18:1}$	3.2	T	13.3	5.2	T	9.9	5.2	6.7	6.0
Linoleic $C_{18:2}$	31.6	44.4	48.0	50.0	33.0	53.4	39.7	25.4	31.6
Linolenic $C_{18:3}$	23.7	T	10.4	20.0	14.5	12.2	15.5	17.0	13.7
Unknown Y	—	—	—	—	T	—	—	—	—
Unknown Z	—	—	—	—	T	—	—	8.7	—

Fatty acid	Days after light treatment											
	3rd				4th				5th			
	D	B	DR	FR	D	B	DR	FR	D	B	DR	FR
$C_{12:0}$	T	—	0.9	T	0.5	T	—	T	2.7	T	—	0.7
$C_{14:0}$	1.6	6.2	2.1	0.8	0.4	T	—	T	T	T	—	T
$C_{14:1}$	4.2	T	—	—	2.7	T	—	—	15.3	T	—	T
$C_{15:0}$(?)	4.0	16.2	1.2	0.2	0.8	1.5	—	T	4.2	1.8	—	—
$C_{16:0}$	40.4	19.9	18.6	26.2	24.3	20.0	38.3	12.4	16.0	29.4	34.4	22.7
$C_{16:1}$	—	22.8	11.1	T	—	3.6	T	T	—	6.4	T	T
$C_{18:0}$	2.4	4.2	3.3	11.0	11.5	3.2	4.4	5.3	2.7	3.2	4.4	2.8
$C_{18:1}$	3.0	3.6	5.0	9.9	14.0	10.1	7.7	8.5	11.3	5.6	4.8	7.6
$C_{18:2}$	32.5	18.1	26.3	32.3	36.0	41.7	30.6	46.0	42.4	34.2	26.4	26.0
$C_{18:3}$	12.2	9.4	23.9	18.8	10.9	20.0	19.0	11.2	5.3	19.4	29.9	36.1
Y	—	—	—	—	—	—	—	5.8	—	—	—	—
Z	—	—	6.0	—	—	—	—	1.7	—	—	—	4.0

T = Trace — Not detectable

The fatty acid composition of the lipids varied with the treatments and changed considerably during the experiment. The major fatty acid components in the shoots were palmitic, palmitoleic, stearic, oleic, linoleic and linolenic (fig. 12). Some fatty acids components such as lauric, myristic, unknowns x, y and z were detected occasionally in some treatments.

The percentage free fatty acid in the oil from the cotyledons of soybean seedlings grown in the dark or under light of different quality changed with the development of the seedlings. There was an increase in percent content of free fatty acids in the oil with development in all cases. The changes in percent free fatty acid were similar in the oil from cotyledons of dark grown, deep-red and far-red treated plants up to the sixth day (fig. 13). The free fatty acids in the oil of cotyledons from the blue were considerably lower for the first five days. On the other hand, by day seven the free fatty acid content in the far-red was much higher than for any of the other treatments.

Although there were 4 to 16% of free fatty acids in the oil as determined by titration (fig. 13), only a trace of a few free fatty acids were detected by GLC (table VII). No free fatty acids were detected by the GLC during the first four days. A few fatty acids were detected in samples from the fifth day onward. The failure to detect free fatty acids by GLC could have been due to the small amount of sample and the method employed to separate the free fatty acids from the aliquots of oil.

The Effect of Light Quality on Protein Content, Amino Acid Composition and Amide Content of Soybean Seedlings

The percentage protein content as determined by total nitrogen (factor 4.93) increased in the cotyledons and the shoots with the development

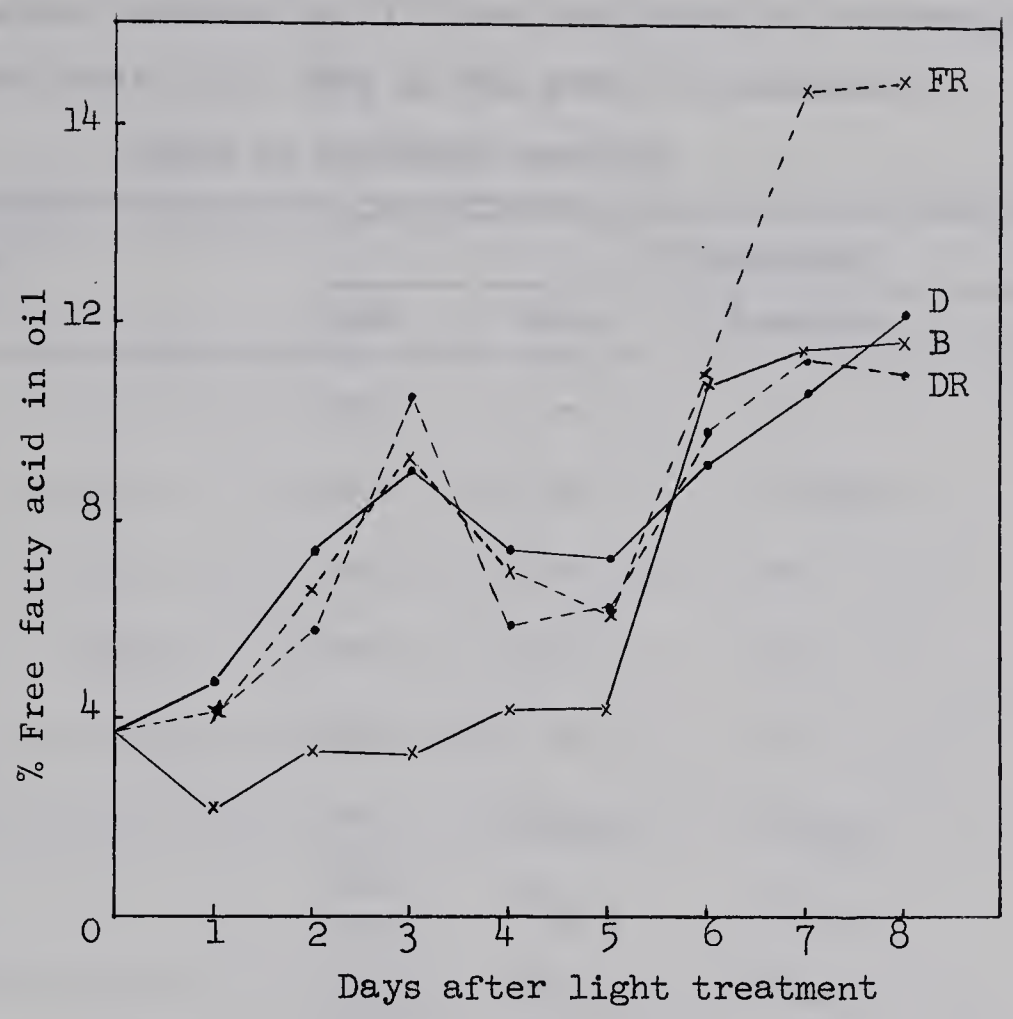


Fig. 13. Free fatty acid content in the oil from the cotyledons of soybean seedlings over the period of 8 days in the dark or under continuous light of different quality. D = dark; B = blue; DR = deep-red; FR = far red.

TABLE VII

Free fatty acids detected in oil from cotyledons of soybean seedlings over the period of 7 days in the dark or continuous light of different quality

Days after light treatment	Treatments			
	Dark	Blue	Deep-red	Far-red
0	—	—	—	—
1st	—	—	$C_{14:0}$	—
2nd	—	—	—	—
3rd	—	—	—	—
4th	—	—	—	$C_{8:0}$
5th	—	$C_{12:0}$	$C_{12:0}$	$C_{16:0}$
6th	$C_{8:0}$ $C_{16:0}$	$C_{16:0}$	$C_{16:0}$	$C_{16:0}$
7th	$C_{16:0}$ $C_{18:0}$ $C_{18:1}$ $C_{18:2}$ $C_{18:3}$	$C_{8:0}$ $C_{16:0}$	$C_{14:0}$ $C_{16:0}$	$C_{14:0}$ $C_{18:1}$ $C_{18:2}$

— Not detected.

of seedlings (fig. 14a, and c). However, in fact, the protein content of the cotyledons decreased drastically as the development of the seedlings proceeded (fig. 14b). This diminution of protein content from the cotyledons was accompanied by an increase in the shoots (fig. 14d). The percentage protein content in the whole seedlings increased gradually (fig. 14e), however, there was essentially no real change in total protein content during the 8-day experimental period (fig. 14f). No striking difference was observed in protein depletion in the cotyledons, or in protein build-up in the shoots, between different light-treated plants (fig. 14b and d). However, after the sixth day of treatment, the protein depletion in the cotyledons of dark-grown seedlings was retarded (fig. 14b). This was accompanied by a less rapid build-up of protein in the shoots (fig. 14d).

Amino acid analyses were done on the cotyledons of soybean seedlings grown under different treatments for 5 days. The results are presented in table VIII for protein amino acids and in table IX for the free amino acids and amides. In the protein of cotyledons from dark-grown plants all amino acids except aspartic were higher than for the light treated plants. On the other hand, the free amino acids (table IX) in the cotyledons of dark-grown plants were slightly lower than in the light treated plants. Among the light treated plants, the protein amino acids in the cotyledons, with a few exceptions, were lowest in the far-red and highest in the blue. The free amino acids in cotyledons of different light treated plants were essentially not different. The cotyledons of dark-grown plants and from different light treated plants were low in free glutamine and high in free asparagine. However, the free asparagine in cotyledons of dark-grown plants was only 50 - 60% as much as that in the light treated plants.

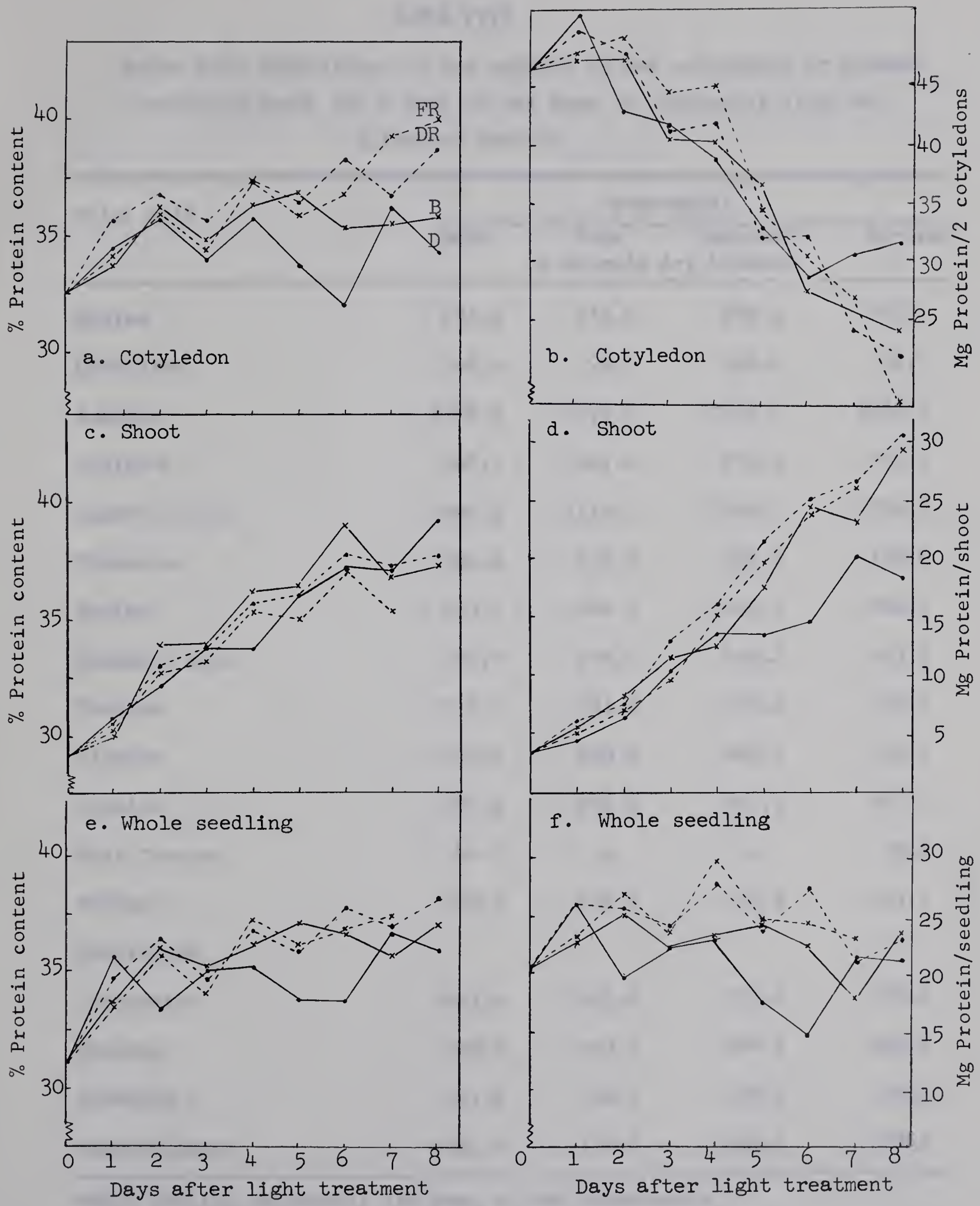


Fig. 14. Protein content of the shoot, cotyledon and whole seedling of soybean during 8 days in the dark or continuous light of different quality. The data are expressed as percentage of dry tissue in a, c, and e, and as weight of protein per plant part or per seedling in b, d, and f.

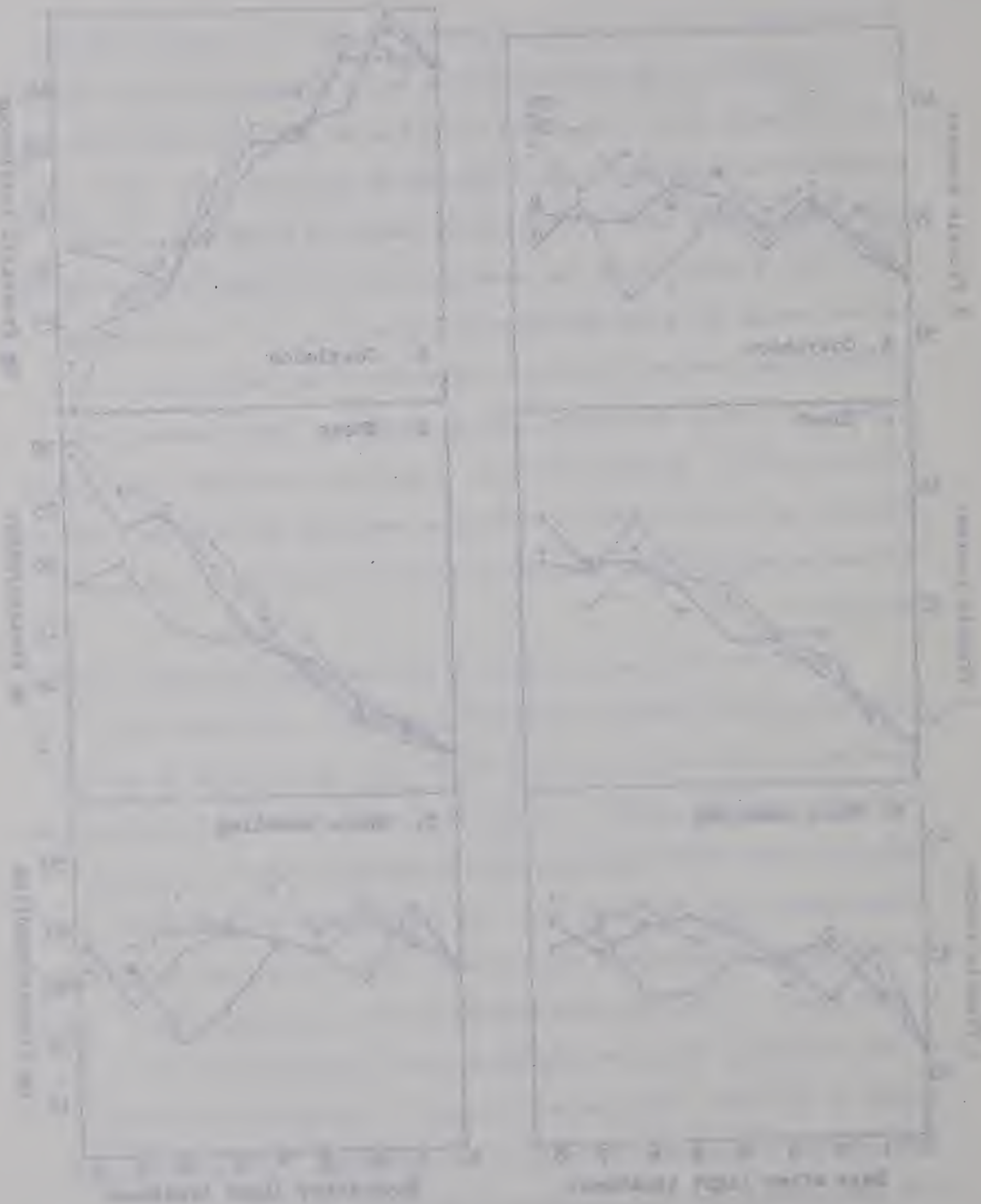


TABLE VIII

Amino acid composition of the protein in the cotyledons of soybean seedlings grown for 5 days in the dark or continuous light of different quality

Amino acid	Treatments			
	Dark	Blue	Deep-red	Far-red
	μ moles/g dry tissue*			
Lysine	231.9	216.6	209.5	202.4
Histidine	102.9	99.1	100.0	92.0
Ammonia	1228.4	1170.2	1295.4	1270.5
Arginine	268.5	227.4	211.5	214.4
Aspartic Acid	906.3	1114.1	1149.1	1216.9
Threonine	182.6	177.6	169.1	162.6
Serine	263.5	244.8	240.2	246.7
Glutamic Acid	556.4	434.6	402.2	383.1
Proline	212.1	181.2	170.6	160.4
Glycine	307.0	280.0	268.5	252.5
Alanine	284.9	256.8	249.5	241.2
Half Cystine	44.7	n	n	32.2
Valine	276.3	232.8	218.9	214.1
Methionine				
Isoleucine	203.2	195.6	171.2	173.4
Leucine	350.9	303.5	284.7	269.6
Tyrosine	117.9	98.7	92.8	85.9
Phenylalanine	191.5	172.8	164.1	158.9

*Each reading represents the mean of two experiments.

n = Not determined.

TABLE IX

Free amino acids and amides in the cotyledons of soybean seedlings grown for 5 days in the dark or continuous light of different quality

Amino acid	Treatments			
	Dark	Blue	Deep-red	Far-red
	μ moles/g dry tissue*			
Lysine	0.07	0.11	0.12	0.09
Histidine	3.28	4.55	3.84	4.09
Ammonia	1.02	1.90	1.60	1.31
Arginine	0.77	1.47	0.86	0.72
Aspartic Acid	0.86	1.52	1.23	1.16
Threonine	3.09	5.42	5.41	4.79
Serine	6.26	9.50	9.71	8.83
Glutamic Acid	6.28	8.97	8.51	6.93
Proline	2.70	4.76	4.27	3.99
Glycine	0.79	1.40	1.10	1.22
Alanine	5.89	9.28	8.71	8.72
Half Cystine	0.41	0.50	0.30	0.38
Valine	4.46	7.21	7.97	6.48
Methionine	n	n	n	n
Isoleucine	2.27	4.07	4.73	3.87
Leucine	1.78	3.08	3.42	3.14
Tyrosine	0.16	0.35	0.42	0.37
Phenylalanine	3.35	6.50	7.62	5.65
Asparagine	38.23	68.41	76.08	62.93
Glutamine	1.30	0.69	3.43	2.28

*Each reading represents mean of duplicate samples.

n = Not determined.

DISCUSSION

According to the present concept of physics, the energy of radiation is contained in packets called quanta. The energy content of the quantum is inversely proportional to the wavelength of the light. The longer the wavelength of light the less energy contained per quantum. Hence the energy content of a quantum of blue light is much higher than for a quantum of red light. With a given energy level, the quantum fluxes are directly proportional to the wavelength of light. As a consequence, the adjustment of light intensity in all the cabinets to a uniform energy level resulted, in fact, in the use of different quantum fluxes in different cabinets.

The development of soybean seedlings under light of different quality or in the dark was distinctly different as shown in figure 4. Measurements done separately on different parts or organs of seedlings showed that the differences in development between different treatments were significant (fig. 3 and 6). The results indicate that light appears to inhibit the elongation of hypocotyl (fig. 3a and fig. 4), but promote the elongation of epicotyl (fig. 3b), the development of root system (fig. 3c and fig. 5), and expansion of cotyledons (fig. 6 and 7) and leaves (fig. 7 and table III). However, results presented in figure 8 show that there was no significant difference in water content of the whole seedling or cotyledons or shoot between different treatments. There were some differences between the dry weight of shoots of dark and light treated plants. In spite of the significant differences in development of cotyledons between different treatments (fig. 6 and 7), no significant differences in dry weight were observed. The increase in volume of the cotyledons of light treated plants was not accompanied by an increase in dry weight, in fact,

a rapid decrease in dry weight of cotyledons with the development of the seedlings was observed (fig. 9a). Thus, the increase in volume of the cotyledonsof light-treated plantsmight have been due mainly to an increase in the cellular spaces in the tissue, similar to that observed in the leaf tissue of Phaseolus vulgaris (Fletcher, Peterson and Zalik, 1965).

As was seen in the results (fig. 7), the cotyledons in soybean seedlings behaved as leaves, that is, they expanded in the presence of light, but responded differently than the leaves to light of different quality. Leaves showed maximum expansion in the deep-red, whereas cotyledons showed maximum expansion in the far-red. Cotyledons from plants grown in the far-red developed morphologically into a leaf-like organ, but with no apparent chlorophyll. The lack of chlorophyll in plants grown exclusively in the far-red has been reported by Withrow, et. al. (1953) for beans and corn. From the results obtained it may be inferred that the greatest expansion of leaves as observed for the deep-red was not due to photosynthesis alone butinvolved a morphogenetic effect of light.

The decrease in dry weight of cotyledons (fig. 9a) and the concomitant increase in dry weight of shoots (fig. 9b) implicates the translocation of reserve food from the cotyledons to the shoots. This indication was further supported by the results from the studies on oil content (fig. 10b and d) and protein content (fig. 14b and d). Although, the dry weight determined for some plant parts (table III) showed significant differences between different treatments, no significant difference was observed in dry weight of the entire shoot with different light treatments. This could be attributed to the fact that the development of different parts or organs of the seedlings under different treatments was compensatory (fig. 3, 4, 5, 6, and 7). The change in dry weight of the shoot

of dark-grown plants followed closely that of the light treated plants for the first 4 days of the experiments. The lag in increase in dry weight of dark grown plants on day 5 was accompanied by the retarded growth of epicotyl and root system in the dark (fig. 3b and c, and table III). This together with the rapid elongation of the epicotyls of light treated plants (fig. 3b) and their greater root elongation (fig. 3c) and better development of roots (fig. 5) resulted in significant less dry weight in the shoots of dark-grown plants (fig. 9b). Morphologically, considerable development was observed in seedlings of all treatments, however, there was no net gain in dry matter in these seedlings during the experiments (fig. 9c). The growth of shoots was probably mainly at the expense of reserve food from the cotyledons. Presumably a gradual decrease in dry weight of the whole seedlings of all treatments was due to loss of respiratory substrate (fig. 9c).

Thomson and Miller (1963) have concluded from their studies with pea that light affects growth of shoots by accelerating all phases of growth and development — division, enlargement, and maturation. The final length of elongation of internodal growth was reduced by light because of accelerated cell maturation and as a result of reduced cell division and elongation in the later phases of growth. In this study, it was found that during the early stage of the experiments there was no difference in elongation of hypocotyl in the light or in the dark. However, the elongation of the hypocotyl ceased earlier in the light than in the dark, with the earliest cessation being in the deep-red (fig. 3a). This resulted in a difference in elongation of hypcotyl under different treatments.

Downs and Cathey (1960) observed that in red kidney bean, active elongation of the epicotyl does not begin until growth of the hypocotyl is nearly complete. The same was observed in this study, as can be seen

from figure 3a and b. Oota, et. al. (1953) also obtained similar results in germination of Vigna sesquipedalis. Similar growth patterns occur in monocotyledons. Stafford (1948) observed that the active growth of the coleoptile and first true leaf of Phleum in both dark- and light-plants is dependent upon cessation of growth of the first internode (mesocotyl). In maize, Inge and Loomis (1937) found that during the normal period of rapid internode elongation, plumule and nodal root development was inhibited. Illumination of coleoptiles, which checked internodal growth, stimulated plumule and root growth. Thus the retarded growth in epicotyl of dark-grown plants observed in this study and by others may be due to the continuous and compensatory growth of the hypocotyl in the dark (fig. 3a and b).

Downs and Cathey (1960) had suggested on the basis of their work that the apparent stimulation of first internode (epicotyl) elongation in bean by light is, most probably not the result of a direct stimulatory effect of the radiant energy upon that internode but is more likely a result of its action on the hypocotyl, hastening its maturity with consequent inhibition of its growth. In view of the fact that different parts and organs of the seedling respond to different quality of light in very different ways, this might be the cause.

Stafford (1948) observed that the cessation of elongation of the first internode (mesocotyl) in Phleum precedes the appearance of pitted xylary elements in the apical zone of this structure, regardless of whether the plants have been grown in the dark or in the light. This indicates that the cessation of elongation is the cause for the onset of maturation of the tissue, but not an effect of accelerated cell maturation in this case. In other words, light causes cessation of the elongation

of the cells in the mesocotyl. From the literature already cited, it can be presumed that light acts in the same way on the inhibition of hypocotyl.

How does light cause the cessation of the elongation of mesocotyl and hypocotyl? It has been observed that the depression in growth by light is always accompanied by a decrease in extractable IAA or lowered IAA activity (Van Overbeek, 1936; Inge and Loomis, 1937; Blaauw-Jansen, 1957; Briggs, 1963). Recently, Fletcher and Zalik (1964) using Phaseolus vulgaris obtained a direct relationship between the IAA content after one light cycle and plant height after 7 cycles. On the basis of the experimental data, Meijer (1958), Fletcher and Zalik (1964) and others have suggested that one of the effects of light on growth is through the regulation of endogenous levels of IAA. One may wonder how the reduction of auxin can be inhibitory to one tissue and be promotive to the other. Thimann (1937) has demonstrated that the sensitivity to auxin is different with different tissues. He observed that the growth of root, bud and stem of pea seedlings are inhibited by relatively high and promoted by relatively low auxin concentrations. The optimum concentration of auxin for promoting growth is highest for stem and lowest for root. In this study, it was found that the deep-red treated plants which had the shortest hypocotyls had the best root development and leaf expansion. On the other hand, the dark-grown plants which had the longest hypcotyls had the poorest root development and retarded plumule growth. The elongation of hypocotyl, root development and leaf expansion in blue and far-red treated plants were intermediate.

Galston and co-workers (Bottomley, et. al., 1965) have shown that light regulates IAA levels through an effect on the synthesis of co-factors or inhibitors of IAA-oxidase which is an enzyme responsible for the destruction of IAA. These co-factors or inhibitors have been identified as phenolic compounds. These effects of light on the phenolic inhibitors and co-factors of IAA oxidase are of interest because it has been found that light also induces or stimulates the synthesis of other structurally related phenolic compounds such as anthocyanin. What seemed especially interesting in connection with this study is that these compounds have been shown to derive from the same pathway as anthocyanin synthesis and acetyl CoA has been known to be a precursor for the synthesis of these compounds (Bottomley, et. al., 1965). This evidence accordingly lends further support to the hypothesis of the Beltsville group that phytochrome action is possibly mediated by CoA.

The changes in lipid content of soybean seedlings (fig. 10e and f) represent a typical curve of oil utilization associated with seedling development as observed in many other oil crops (Boatman and Crombie, 1958; White, 1958; Brown, et. al., 1962; Huber and Zalik, 1963). The results indicate that the degradation of oil is probably restricted mainly to the cotyledons. Since the cotyledons are the storage organs of the seeds, such a restriction may be expected during rapid growth of young seedlings. The gradual decrease in the percent oil content of the shoot (fig. 10c) and the accompanying actual increase in total lipid content (fig. 10d) with the development of the seedlings could be attributed to an increase in fatty materials such as phospholipids of cell membrane, and waxes, sterols and other fat soluble materials, but at a lower rate than cell wall and proteinaceous materials (fig. 14d).

The rapid decrease in oil content in the cotyledons indicates the utilization of reserve fat for growth. In plants the reserve fat will be hydrolyzed to the component fatty acids and glycerol through the activity of lipase. Fatty acid degradation in plants mainly takes place through β-oxidation and results in the production of acetyl CoA. In germinating seeds or developing seedlings it has been demonstrated (Carpenter and Beevers, 1959) that acetyl CoA can be converted to carbohydrate through the glyoxalate cycle. In this study, the rapid diminution of oil from the cotyledon and the accompanying increase in non-lipid matter in the shoot suggests the involvement of the glyoxalate cycle.

The results obtained in this study indicate that light accelerated the rate of lipid utilization in soybean seedlings (fig. 10b and table IV). A greater utilization of fat during germination in the light than in the dark has been reported by MacLachlan (1936) for soybean, and by White (1958) for cotton seeds. Mohr (1965), using mustard seedlings also found that far-red light increased the rate of fat degradation in the cotyledons as compared with darkness. Light of different quality apparently affected oil degradation in cotyledons of soybean seedlings differently. In this study, far-red was found to be more effective than blue and deep-red.

It may be inferred from the work of Carpenter and Beevers (1958) that light probably exerts its effect on oil degradation through accelerating the enzyme activity of the glyoxalate cycle. The diminution of fat from the cotyledons was found to parallel the expansion of cotyledons (fig. 7 and table IV). Whether there is a causal relationship between these two observations is not known. No other growth response showed any correlation to the rate of fat diminution in the cotyledons. As shown in figures 3, 4, 5, and 7, the greatest leaf expansion and best root

development occurred in the deep-red and the longest hypocotyl was found in the dark. The dry weight of shoots under different light treatments was essentially not different. From these results it might be concluded that light of different quality has different effects on oil depletion in the cotyledons independent of the morphogenetic effects.

The difference in oil content between the shoots of different treatments could be attributed to the difference in growth and development of these plants under different treatments. The lower oil content in the shoot of dark-grown plants as compared to that in the light in the later stage of development is probably due to the retarded growth of the plumule and poorer root development. On the other hand, the higher oil content in the shoot of deep-red and blue treated plants than that of the far-red may have been due to the formation of chloroplasts and greater leaf surface and thus more fatty materials such as waxes and sterols and fat soluble materials such as pigment.

Surprisingly, the fatty acid composition of the oil from cotyledons and embryos of the parent seeds was different. In addition the percent composition of some of the acids common to both tissues varied. A review by Shorland (1962) drew attention to the fact that fatty acid composition varies with different organs or tissues of the plant.

Fatty acid analyses of the oil from cotyledons showed that during eight days of development there was little change in fatty acid composition. Since the oil content in the cotyledons decreased during this period of time the fatty acids must have been utilized more or less in proportion to the amount originally present. MacLachlan (1936) found that there was no preferential utilization of unsaturated or saturated

fatty acids in germination of soybeans because no change was observed in the average unsaturation of the fatty acids. Holman (1948), however, also using soybean, reported a decrease in the iodine value of the fat reserve and preferential utilization of linoleic and linolenic acid. On the other hand, Brown, et. al. (1962) reported a slight but continuous increase in the iodine value of the natural fat of soybean cotyledons during the 12-day germination period in the dark and a relatively faster depletion of oleic than the other fatty acids. They attributed the difference between their observation for iodine number and those of Holman to the fact that Holman's measurements may have been affected by the increasing proportion of non-triglyceride matter in crude fat from the whole seedling. In this study, in general, a slight but continuous increase in linoleic acid accompanied by a decrease in oleic was observed in oil from all treatments in the early stage of the experiments. There was also a decrease in linoleic accompanied by an increase in linolenic acid from the fifth day onward (fig. 11). However, due to the differences in experimental techniques, the results may not be strictly comparable.

Although, there were differences between treatments in the utilization of fat there was almost no difference in the percentage of various fatty acids in the neutral oil from cotyledons of different treatments. Huber and Zalik (1963) working with flax, and White (1958), working with cotton seed also found no apparent differences in the fatty acid composition due to illumination. It should be pointed out that the oil in the cotyledons was predominantly in the form of triglycerides and the method employed for this portion of the study involved tranesterification of the fatty acids in the glycerides. As a consequence, little changes in

fatty acid composition would be expected, unless the activity of lipase was selective. Changes in fatty acid composition of the oil might have occurred in the free fatty acid portions if there was preferential utilization of particular fatty acids. Unfortunately, the results obtained for the free fatty acid analyses failed to adequately test this possibility, perhaps, due to the inadequacy of the analytical method. Although 4 - 16% of free fatty acid was present in the oil as determined by titration, almost no fatty acid was detected by GLC in the samples from 0-4 days treatment. The few fatty acids detected in samples taken from the fifth day onward were mainly palmitic and other saturated fatty acids. Since these results were based on one experiment and the titration and GLC data were not in agreement conclusions from these might not be valid.

With the development of the seedlings there was a rise in the percent of free fatty acids in the oil of cotyledons. In his studies on cotyledons of cotton seedlings, White (1958) also observed that free fatty acids rose from 0.5% to 20% during seven days of germination. However, the actual amount of free fatty acids remained relatively constant. Brown, et. al. (1962) also obtained similar results for soybean. The rise in percent free fatty acid content with the development of the seedlings as observed in this study and by others was probably due to the continuous diminution of the oil from the cotyledon. On the other hand, the actual constant amount of free fatty acid might be explained by an interrelationship or balance between lipase activity and β-oxidation. The difference in changes in percent free fatty acid content between different treatments might also be explained by the foregoing (table IV and fig. 13).

The composition of the oil from the shoot was markedly different than that of the cotyledons, both in percentage and types of fatty acid .

It has been pointed out by Shorland (1962) that the nature of the lipids from different parts of plants are different. In the seed fats, the lipids are largely triglycerides whereas in the leaf, the lipids contain mainly galactolipids in place of triglycerides, the remainder consisting of unsaponifiable matter, waxes and phospholipids. The leaf fats are not necessarily related to the seed fats in their fatty acid composition. As shown by the fact that fruit-coat and seed fats each elaborates it own types of fatty acids. Boatman and Crombie (1958) observed that the fatty acid composition in the root of West African oil palm is totaly different from that of the shoot.

The changes in percent distribution of fatty acids of the shoot in this study were erratic and were markedly different between different treatments. This variability might be explained by one of the following considerations. As the results were calculated in percent fatty acid composition a change in any of the components would cause changes in all the other components. Boatman and Crombie (1958), also observed similar fluctuations in percent distribution of fatty acids in their work with palm. But when the results were converted to amounts by weight the fluctuations were greatly reduced. In this study, such determinations were not made because there were considerable quantities of pigment present in the samples from the blue and deep-red treatments, but not the far-red and dark. It was observed that different parts of the shoot developed differently under different light treatments, but the fatty acids analyses were done for the shoot as a whole. If the different plant parts, in fact, have their own type of lipids and fatty acids and if the differences in development were accompanied by distinct changes in fatty acid composition then what seems like erratic data for fatty acid

changes in the shoot might result (fig. 12). Further detailed study of different classes of lipid in different parts of the plant might elucidate the possible biological significance of these differences in relation to the morphogenetic responses of the seedlings under different treatments.

During the development of the seedlings there was a rapid diminution of nitrogenous material from the cotyledons accompanied by a rapid increase in the shoots (fig. 14b and d). Similar results were also observed for germination of barley by Folkes and Yemm (1958), for corn by Inge, Beever and Hageman (1964) and for Vigna sesquipedalis by Oota, et. al. (1953). The proteins in cotyledons are probably being broken down to the component amino acids which are then translocated. Folkes and Yemm (1958) observed that when barley grains were germinated in the absence of external supplies of nitrogen, their metabolism of amino acids and protein was dominated by the transformation of reserves stored in the endosperm, into protoplasmic protein and other nitrogenous constituents of the young embryo.

The slight but continuous increase in percent protein content of the cotyledons accompanied by a rapid decrease in the actual amount of protein in them was probably due mainly to a slower utilization of nitrogenous reserves than lipid.

Light did not seem to have any direct effect on the diminution of nitrogenous materials from the cotyledon (fig. 14b) but may have affected protein transformation in the cotyledons as implied by the amino acid data. The difference in deposition of nitrogenous materials in the shoot between light and dark treatments in later stages of the experiment appeared to be due to differences in growth. As might have

been expected there was essentially no change in the total nitrogen during the experiment.

Analyses of amino acids and amides in cotyledons after five days treatment (table VII and VIII) showed that dark-grown seedlings were higher in protein-bound amino acids and lower in free amino acids than cotyledons from the light treated plants. This indicates that light accelerates the breakdown of the proteinaceous compounds.

Protein-bound amino acids were least in cotyledonous tissue from far-red and essentially there was no difference in amount for different components between blue and deep-red. This implies that the depletion of proteinaceous compounds was more rapid in far-red than in other treatments.

Striking differences in amounts of certain active amino acids such as glutamic and aspartic, and the amides asparagine and glutamine, between tissues of different treatments, were observed. The interconversion of one from the other or a by-pass of one of these to other amino acids has been reported. However, in the present study no attempt was made to trace these changes under different treatments. Nevertheless, the results obtained suggest that this is worth further investigation.

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